" A COMPARATIVE EVALUATION OF EFFECT OF HORMONE REPLACEMENT THERAPY VERSUS RALOXIFENE ON CLINICO-BIOCHEMICAL, HISTOPATHOLOGICAL CHANGES AND BONE MINERAL DENSITY IN POST MENOPAUSAL WOMEN "

THESIS

FOR

MASTER OF SURGERY

(OBSTETRICS AND GYNAECOLOGY)

M.L.B. MEDICAL COLLEGE

BUNDELKHAND UNIVERSITY

JHANSI (U.P.)

2003

JAYA PATHAK

Certificate

*This is to certify that the work entitled, "**A Comparative evaluation of effects of Hormone replacement therapy and Raloxifene on Clinico-biochemical, histopathological changes & bone mineral density in post menopausal women**" which is being submitted as a thesis for Master of Surgery (Obstetrics and Gynaecology) by Dr. Jaya Pathak, has been carried out in the Department of Obstetrics and Gynaecology, M.L.B. Medical College, Jhansi.*

She has put in the necessary stay in the department as per university regulations.

Dated : 23.4.2013

Dr. M. Kapoor
M.S.
Professor and Head,
Department of Obstetrics & Gynaecology,
M.L.B. Medical College, Jhansi

Certificate

This is to certify that the work entitled, ***"A Comparative evaluation of effects of Hormone replacement therapy and Raloxifene on Clinico-biochemical, histopathological changes & bone mineral density in post menopausal women"*** *which is being submitted as a thesis for Master of Surgery (Obstetrics and Gynaecology) by Dr. Jaya Pathak, has been carried out in the Department of Obstetrics and Gynaecology, M.L.B. Medical College, Jhansi.*

The results and observations recorded have been periodically checked and verified by me.

Dated : 23.4.2003

Dr. Sunita Arora
M.S., F.I.C.O.G.
Professor
Department of Obstetrics & Gynaecology,
M.L.B. Medical College, Jhansi

Certificate

This is to certify that the work entitled, ***"A Comparative evaluation of effects of Hormone replacement therapy and Raloxifene on Clinico-biochemical, histopathological changes & bone mineral density in post menopausal women"*** *which is being submitted as a thesis for Master of Surgery (Obstetrics and Gynaecology) by Dr. Jaya Pathak, has been carried out in the Department of Obstetrics and Gynaecology, M.L.B. Medical College, Jhansi.*

The results and observations recorded have been periodically checked and verified by me.

Dated : 23.4.2003

Dr. S. Sharma
M.D.
Associate Professor
Department of Obstetrics & Gynaecology,
M.L.B. Medical College, Jhansi

Certificate

*This is to certify that the work entitled, "**A Comparative evaluation of effects of Hormone replacement therapy and Raloxifene on Clinico-biochemical, histopathological changes & bone mineral density in post menopausal women**" which is being submitted as a thesis for Master of Surgery (Obstetrics and Gynaecology) by Dr. Jaya Pathak, has been carried out in the Department of Obstetrics and Gynaecology, M.L.B. Medical College, Jhansi.*

The results and observations recorded have been periodically checked and verified by me.

Dated : 23.4.2003

Dr. R.C. Arora
M.D.
Professor & Head
Department of Medicine
M.L.B. Medical College, Jhansi

Certificate

This is to certify that the work entitled, ***"A Comparative evaluation of effects of Hormone replacement therapy and Raloxifene on Clinico-biochemical, histopathological changes & bone mineral density in post menopausal women"*** *which is being submitted as a thesis for Master of Surgery (Obstetrics and Gynaecology) by Dr. Jaya Pathak, has been carried out in the Department of Obstetrics and Gynaecology, M.L.B. Medical College, Jhansi.*

The results and observations recorded have been periodically checked and verified by me.

Dated : 23.4.2003

Dr. Navneet Agarwal
M.D.
Associate Professor
Department of Medicine
M.L.B. Medical College, Jhansi

Certificate

This is to certify that the work entitled, ***"A Comparative evaluation of effects of Hormone replacement therapy and Raloxifene on Clinico-biochemical, histopathological changes & bone mineral density in post menopausal women"*** *which is being submitted as a thesis for Master of Surgery (Obstetrics and Gynaecology) by Dr. Jaya Pathak, has been carried out in the Department of Obstetrics and Gynaecology, M.L.B. Medical College, Jhansi.*

The results and observations recorded have been periodically checked and verified by me.

Dated : 23.4.2003

Dr. Ratna Saxena
M.D.
Professor
Department of Pathology
M.L.B. Medical College, Jhansi

Certificate

*This is to certify that the work entitled, "**A Comparative evaluation of effects of Hormone replacement therapy and Raloxifene on Clinico-biochemical, histopathological changes & bone mineral density in post menopausal women**" which is being submitted as a thesis for Master of Surgery (Obstetrics and Gynaecology) by Dr. Jaya Pathak, has been carried out in the Department of Obstetrics and Gynaecology, M.L.B. Medical College, Jhansi.*

The results and observations recorded have been periodically checked and verified by me.

Dated : 23.4.2003

Mr. B.D. Mathur
M.Sc., D.H.E.
Professor
Statistics & Demography
Department of Obstetrics & Gynaecology,
M.L.B. Medical College, Jhansi

Acknowledgement

"To teach, to guide
to explain, to nurture -
these are life's
noblest attainments"

With first thought I offer my obeisance to God almighty without whose silent but omni present help none of this would have been possible.

I express my utmost, heart felt acknowledgement to my respected teachers, who embody vast knowledge, experience and perform the task of God by imparting knowledge to us and moulding our future.

With a sense of reverence and humble submission , I am very grateful to Dr. M. Kapoor (M.S.) , Professor & Head, Department of Obstetrics & Gynaecology, M.L.B. Medical College, Jhansi, also my co-guide for moulding me capable enough to understand the subject and imparting the knowledge and skill necessary for this study. Her ever helpful nature, generosity, loving attitude & practical approach to life will always be a source of inspiration to me. I shall always remain indebted to her guidance, advise and help

It is with a profound sense of gratitude and regards that I express my thanks to my guide Professor Dr. Sunita Arora (M.S., FICOG), Department of Obstetrics and Gynaecology. Her keen interest, untiring efforts, constant supervision, constructive criticism and valuable help which she readily extended to me at every stage of this work has been a constant source of encouragement.

Words fail to express my gratitude to my co-guide, Dr. S. Sharma M.D., Associate Professor, Department of Obstetrics and Gynaecology , M.L.B. Medical College, Jhansi. Her invaluable suggestions, silent yet strong attitude towards work were a constant source of inspiration during the course of this work.

I am grateful to my co-guide, Dr. R.C. Arora (M.D.) , Professor & Head of the Department of Medicine, M.L.B. Medical College, Jhansi for providing me all facilities and rendering his valuable guidance & supervision. His wide experience in the field of medicine has helped me to accomplish the present work with unobstructed spontaneity.

Also, I am extremely grateful to Dr. Navneet Agarwal (M.D.) Associate Professor, Department of Medicine, M.L.B. Medical College, Jhansi my co-guide, for his guidance and keen interest extended to me during this period of study.

It is with due regards that I pay my gratitude to my co-guide Dr. Ratna Saxena (M.D.), Professor, Department of Pathology for her support and knowledge of various aspects of the subject. It would have been difficult for me to accomplish the project without her help.

I am extremely grateful to Mr. B.D. Mathur , M.Sc., D.H.E., Professor , Statistics and Demography, Department of Obstetrics and Gynaecology, M.L.B. Medical College, Jhansi for his keen interest in this work and his ready help in the completion and evaluation of monumental statistics during the course of this work.

I shall forever, remain grateful to my revered teachers, Dr. U. Agarwal (M.S.), Professor , Department of Obstetrics and Gynaecology and Dr. S. Kharkwal (M.D.) Associate Professor, Department of Obstetrics & Gynaecology, M.L.B. Medical College,

Jhansi for providing me with help and guidance at every stage of this work.

I also like to thank Dr. Praveen Jain (Radiologist - Vardhman Diagnostic Centre, Jhansi) for providing me with help as and when required.

I also wish to record my heartfelt appreciation for immense cooperation of all the residents of Obstetrics and Gynaecology department.

Words can not express the debt I owe to my mother. She has always been there to encourage and motivate and make me what I am. To my father, whose soul lies within me, I owe everything, though he has departed from this world. I can always feel him watching from the heaven above, showering his blessings in every step of my life. I thank my father in law and mother in law for all the concern and encouragement they have given me without which I would not have been here.

My heartly thanks to my husband, Dr. Rishi for his ever encouraging and helpful attitude. Without his support, patience and constant guidance, the present work would not have been possible. His mere presence has motivated me to attain the goals which seemed impossible.

I am thankful to Mr. Rajendra (Priyanka Computer) for typing the script of this text and giving a beautiful shape to my thesis.

Finally, I wish to thank my patients who contributed and cooperated without which this work would have never reached the present stage.

Jaya Pathak.

Dated : 23.4.2003

Jaya Pathak

Contents

- Introduction 01 - 03
- Aims & Objective 04
- Review of Literature 05 - 49
- Material & Method 50 - 61
- Observation 62 - 78
- Discussion 79 - 88
- Summary & Conclusion 89 - 95
- Bibliography 96 - 108
- Master Chart

Introduction

INTRODUCTION

Menopause is defined as permanent cessation of menstruation at the end of reproductive life due to loss of ovarian follicular activity, It is a natural and inevitable phenomenon and serves as a natural event.

Climacteric is the physiologic period in women's life during which there is regression of ovarian function.

When Menopause is induced it is known as artificial or induced menopause. It is induced artificially by surgical removal of both ovaries called SURGICAL MENOPAUSE or by suppressing ovarian function by external gamma radiation , or intracavitary radiation called radiation induced menopause.

Menopause occurs at median age of 51 years (according to western data). In India , age of menopause is 44-50 years. It occurs earlier in cigarette smokers, nulliparous women, high altitude inhabitant. It appears to be genetically predetermined. But approximately 1% of women undergo menopause before the age of 40 years known as premature ovarian failure ***(R. Barbee, H Abdulla, J Studd Vol. 9, 209)***.

The women of post menopausal group suffer from symptoms of vasomotor instability (i.e. hot flushes, night sweats, palpitations, insomnia etc)., urogenital atrophy (i.e., vaginal dryness, dyspareunia, itching, urinary incontinence,

frequency, urgency, nocturia and dysuria etc), Psychosomatic changes (i.e. irritability, depressive symptoms, insomnia, diminished libido etc). and late consequences such as bone pain, spontaneous fracture, cardiovascular diseases and Alzheimer disease. The young age of artificially induced menopause causes abrupt onset of these symptoms as compared to natural menopause.

Numerous studies suggest that lack of ovarian function is responsible for altered lipid profile, which increases the risk of coronary artery diseases, and osteoporosis via altering calcium metabolism in both natural menopause or induced menopause.

Not so long ago women viewed menopause as a natural process of aging, to be tolerated with static silence. Over a past few decades, we have achieved significant progress in our understanding of menopause and have come to view this period of life more and more as a state of estrogen deficiency.

Current demographic trends indicate that due to increase in life expectancy and increased incidence of panhyste-rectomies at an early age for various gynecological causes, about 1/3rd of women's life is in her post menopausal period. On the other hand, women are now playing increasingly active roles in the social and professional areas. So improving quality of life during post menopausal years has grown to be an important issue.

Estrogen deficiency causes quality of life to be poor at and after menopause. Estrogen can be given by a variety of routes- oral, transdermal, subcutaneous, vaginal.

It has become abundantly clear that patients, who are on long term estrogen replacement therapy are at lower overall mortality risk than women who do not receive any replacement. However, benefit of HRT needed to be weighed against possible risk associated with long term use such as uterine and breast cancer and thromboembolic phenomenon.

Selective Estrogen Receptor Modulators are the new class of drugs which is believed to have ideal properties for a product designed for menopausal women. One most widely studied member of this class is RALOXIFENE. It is a nonsteroidal derivative of benzothiophene.

Accumulating evidence is there from many studies that SERMS - Raloxifene may confer health benefits related to cardiovascular disease, osteoporosis, endometrial cancers.

The present study is conducted to compare the effects of estrogen replacement therapy and Raloxifene in post menopausal women of Bundelkhand region.

Aims & Objectives

AIMS & OBJECTIVES

The present study is aimed at -

1. Assessing the changes in clinicobiochemical and histopathological parameters and bone mineral density following administration of raloxifene in postmenopausal women.
2. Assessing the changes in clinico-biochemical, histopathological parameters and bone mineral density following administration of estrogen replacement therapy in post menopausal women.
3. Comparing the effects of raloxifene and estrogen replacement therapy in postmenopausal women.

Review of Literature

REVIEW OF LITERATURE

Menopause defined as the permanent cessation of menses that occurs after the cessation of ovarian function, is one event rather than a period of time. It is a natural event – a part of the normal process of aging.

In 1842, ***Brerre de Beusmart in "Al La Menstruation"*** discussed many aspects of menstruation, He was the 1st to discuss about menopause and according to him, it is the termination of reproductive phase of life in women.

Menopause is merely one milestone in this highly complex life cycle of women. It can be spontaneous or surgical and marks a new stage in women's life ***(Speroff et al 1994)***. In cases of surgical menopause, young age of these women compared with those undergoing natural menopause and the abrupt onset of associated symptoms create special problems ***(Wilcox et al 1988)***.

As per the ***WHO technical report series '670'***, the term menopause is defined as the permanent cessation of menstruation resulting from loss of ovarian follicular activity. Women are considered to be menopausal if menses have ceased because of removal of both ovaries along with uterus or for women with intact uterus, menses have ceased at least for a period of one year period after the age of 40 years ***(Rocenberg et al 1993)***.

Since 1960's , a considerable data has been accumulated regarding human climacteric. Climacteric is that phase of aging process of women which marks the transition from the reproductive stage of life to non reproductive stage. It can be regarded as a specific pathologic process or as an endocrinopathy ***(Utian, 1980)***.

Age of Menopause

In developed countries median Age of menopause is 50 year ***(Brambrilla DJ et al, 1989 ; Mc kinlay Sm et al, 1992)***. Some studies done in India have reported the variable data regarding the age of menopause from 42.55 years to 48.62 years ***(Wyan et al 1966, Sharma et al 1980, Randhawa et al 1987)***.

The age of menopause appears to be genetically determined. It occurs earlier in cigarette smokers, in nulliparous women, and those undergoing hysterectomies ***(Siddle N et al 1987, Brambilla DJ et al 1989)***.

Physiology of Menopause

Menopause is a hormone deficient state where ovarian activity ceases. No corpus luteum is formed, no progesterone secreted by ovary and there is no menstrual cycle. All ovarian activity also diminishes and atrophic ovaries lead to menopause.

Due to cessation of follicular development, there is a drop in estradiol level causing loss of negative feed back on hypothalamic pituitary centre which, in turn, is responsible for the increase in FSH and LH by anterior pituitary ***(Yen, 1979)***. With further advancing years, gonadotrophin activity of anterior pituitary ceases. Hence, there occurs a fall in FSH & LH ***(Thaws, 1995)*** Thus, menopause is a hypoestrogenic state, estrogen being secreted in very small amounts, the source of which is the adrenal cortex, ***(Langcope, 1971)***.

Symptoms & Physical Changes

This estrogen deficient state may provoke a variety of metabolic , endocrinal , biochemical, structural and symptomatic alterations in postmenopausal women which may be termed as post menopausal syndrome.

According to ***Lengaten & Kraines (1966)***, symptoms of menopause may begin in perimenopausal period, maximum of complaints, usually 2-3 years after menopause and then slowly decrease. There may be short term and long term complications. While short term complications should be treated, long term complications must be prevented.

Early Symptoms

Include symptoms of Vasomotor instability i.e. Hot flushes, Night sweats, Vertigo, Palpitations , weakness etc.

Intermediate Symptoms

a) ***Urogenital atrophy*** :- Such as atrophy of vagina , bladder and uterus etc. Symptoms produced are vaginal dryness, pruritus, discharge, dyspareunia and urethral syndrome such as stress incontinence, burning, frequency, urgency of micturition etc.

b) ***Psychosomatic Changes*** :- Anxiety, irritability, depressive symptoms, insomnia, sexual changes, diminished libido.

Late Symptoms

a) Osteoporosis, presenting as bone pains and spontaneous fracture.

b) Cardiovascular complications.

Early Symptoms

Hot flushes - It is the classic symptom associated with estrogen deficiency. It is described as "recurrent , transient periods of flushing, sweating, and a sensation of heat, often accompanied by palpitations, feeling of anxiety and sometimes followed by chills" ***(Kroenberg F, 1990)***. It is felt most commonly on the face, the arms and on upper part of body. It may associated with insomnia or sleep disturbances. It is often the earliest and commonest symptom presented in 60-80% of perimenopausal and post menopausal females. The entire episode lasts usually no more than 1-3 minutes, and may

recur as many as 30 times a day, although 5-10 times per day is more common. During the episode, temperature may rise by 5°C. These flushes are accompanied by vasodilatation & elevation of lutenizing hormones. They are experienced more often after surgical menopause. ***(Weinstein L 1990 ; Kronenberg F 1990)***

Physiologically, they correspond to marked, episodic increase in the frequency and intensity of GnRH pulse from the hypothalamus. Instead, the increased pulsatile activity is a marker for some central disturbance of the body temperature regulation center which is responsible for the hot flushes ***(Ravnikar V. 1990)*** Symptoms of vascular instability subside by itself within 3-5 years (Kronenberg F, 1990).

Intermediate Symptoms

Urogenital Atrophy

Atrophic Changes occur with greater severity in tissues with preponderance of estrogen receptors i.e. vaginal tissue, uterus, distal urethra & trigone of bladder. Recent studies have found hormone receptors in females in pelvic musculature like levator ani and ligaments like round ligament ***(Smith et al 1990)***. Within 4-5 years, approximately one-third of women develop symptomatic atrophy. ***(Notelovitz M, 1978)***.

Decreased estrogen levels result in thinning of vaginal epithelial cells, they remain immature, glycogen content

decreases and pH increases. According to ***Raz R., Stamm W E, (1993)***. vaginal symptoms include vaginal dryness, dyspareunia, recurrent vaginal infections. Fortunately, these symptoms are reversible with estrogen therapy.

According to ***Houser et al, 1981,*** Vulval atrophy is sometimes accompanied by pruritis and burning is there.

Urinary symptoms may include dysuria, urgency, frequency, recurrent urinary tract infections, nocturia etc. ***(Notelovitz M, 1989)***. In addition, genuine stress incontinence may be related to estrogen deficiency. As estrogen receptors are found in distal urethra, post menopausal changes lead to atrophy of mucosa, decreased vascularity, diminished tone of urethral musculature which result in recurrent attacks of UTI and stress incontinence ***(Reckess et al, 1992)***. Estrogen therapy may improve or cure stress incontinence in more than 50% of treated women (Bhatia NN et al 1989).

Psychological Symptoms –

Psychological Symptoms such as anxiety, irritability, depressive symptoms and insomnia are most common, just before the onset of menopause.

Falling estrogen levels are directly related to mood changes and psychosomatic symptoms. Vasomotor symptoms often lead to sleep deprivation, chronic fatigue and hence related to psychological symptoms such as depressive symptoms, irritability and mood changes. Women often

experience difficulty in concentrating and loss of short term memory which may be attributed to aging alone or may be due to subtle sleep deprivation associated with hot flushes and replacement therapy with estrogen has been shown to improve both short term memory and psychological function in post menopausal women ***(Dennerstein et al , 1979 ; Ditkoff EG et al, 1991)***.

LONG TERM COMPLICATIONS

Cardiovascular Disorder

Menopause increases the risk of coronary artery disease due to adverse changes in serum lipids and lipoprotein levels and declining estrogen level. The risk of coronary artery disease is at least three times as great for men as for women before menopause and the relative risk for women increases significantly after menopause.

Lobo RA 1990 has shown that estrogen deficiency significantly increases the risk of cardiovascular disease and this can be reduced by estrogen replacement therapy.

Osteoporosis

By definition, osteoporosis is the reduction in quantity of bone. The etiology of osteoporosis is multifactorial. Main factors are age, heredity, estrogen status. Dietary calcium is the most important factor associated with bone loss. Before menopause, the rate of loss is less than 1% of total bone

tissue per year. After menopause, rate of bone loss increases to as high as 5% per year in estrogen deficient women ***(Riggs BL, 1987) Peck WA (1990).*** Sudden decrease in estrogen levels as seen after oopherectomy or with the onset of amenorrhoea is associated with dramatic changes in remodeling of bone resulting in decrease in trabecular bone lead to an increased predisposition for spontaneous fracture. Symptoms produced are body pain, backache, decrease of height, kyphosis, wrist fracture after minor trauma and spontaneous fracture of long bones.

Other Symptoms

Skin Changes – Estrogen receptors are also found in skin & collagen tissue. After menopause, due to decrease in estrogen, skin becomes dry and wrinkled. Due to increased production of melanin, complexion becomes dark.

Hormonal Changes following Menopause

In human ovary, there is continuous and progressive decline in the number of follicles. This loss can not be accounted for by ovulation alone. All types of follicles small, medium, large show decline in number with age continuously through a process of atresia also.

Progressive decline in number of ovarian follicles is responsible for decreased production of ovarian Inhibin ***(Mc Lachlan et al 1988)***. Ovarian Inhibin is a non steroidal,

water soluble protein secreted by granulosa cells of graffian follicle under the influence of estrogen. It suppresses the pituitary follicle stimulating hormones by negative feed back mechanism and ultimately FSH decreases in blood.

In menstruating women, FSH, on day 3 of cycle should be 5-10 IU/L with normally functioning ovaries Elevated FSH levels >40 IU/L are consistent with complete cessation of ovarian functions.

Prior to menopause , LH levels are in the range of 5-10 IU/L. LH levels increase in the menopausal transition in a manner similar to FSH.

First detectable endocrine manifestation is a gradual increase in plasma follicle stimulating hormone. Sometimes, after the rise of FSH, estradiol level decrease slightly and serum LH increases. Eventually as estradiol secretion falls to very low level, both FSH & LH rise in post menopausal period and remain elevated.

Hill (1967) showed that physiological symptoms in artificial menopause are not more serious than in normal menopause if the women has already reached the age of this change.

Hot flushes are apparently the result of instability between hypothalamus and autonomic nervous system brought about by a decline in estrogen. They are particularly

disturbing at night, perhaps because the hypothalamus is in relative state of rest.

Hot flushes are the pathognomic symptom of menopause and the one that most frequently prompts the post menopausal women to seek medical care ***(Maschak et al 1985 ; Erlik et al 1981)***.

Young et al (1957) observed that untreated menopausal women may experience dysuria due to senile urethritis caused as a result of atrophy of bladder and urethral mucosa. ***Rud et al (1980)*** concluded that the subsequent alteration in urethral and bladder pressure can cause urgency, stress incontinence and frequency. A variety of complaints such as headache, insomnia, myalgia, and change in libido may also be noted. The last but not the least are the health problems secondary to deprivation of estrogen which include the osteoporosis and cardiovascular disease.

Riggs et al (1969) observed that declining level of estrogen after menopause leads to an increased rate of bone resorption and greater urinary excretion of calcium. Women remain asymptomatic for 10 years after menopause but lost 15% of their bone moss, setting the stage for fractures.

The precise understanding of the symptom complex which the patient display is often difficult to achieve. Some patients will experience severe multiple reactions which are disabling while others show no reaction or minimal reactions

which go unnoticed until careful medical evaluation is done. Symptoms typically appear when plasma estradiol concentration falls below 35pgm/ml and 40% of women develop menopausal symptoms serious enough to seek medical assistance ***(Carr & Wilson, 1987)***.

Campbell & Whitehead (1977) have concluded from both long term and short term trials that many symptomatic improvement ascribed to estrogen therapy results from relief of hot flushes, improvement in memory and reduction in anxiety. ***Schiff et al (1979)*** showed that estrogen therapy improves the quality of sleep, decrease the time of onset of sleep and increases the rapid eye movement and sleeptime.

Smith et al (1976), Rud (1980), Hiltron et al (1983) concluded that use of exogenous estrogen either prevents or relieves urinary symptoms of menopause.

Semmens et al (1982) have demonstrated that vaginal factors which influence the enjoyment of sexual intercourse can be maintained by appropriate dose of estrogen.

Brinent et al (1985) observed that the collagen content in the dermis of women on exogenous estrogen therapy was maintained at the premenopausal levels. Also estrogen replacement therapy not only prevents loss of bone mass but also reduces the risk of fractures ***(Ettiniger et al , 1985 and weiss et al, 1980)***.

Risk factors for Cardiovascular Disease

Some risk factors for cardiovascular disease include elevated blood pressure, impaired blood glucose tolerance, abnormal blood lipid levels, cigarette smoking, male sex and advanced age. Elevated blood pressure and alteration in lipid and lipoprotein metabolism are major contributors to cardiovascular disease in general and coronary heart disease in particular ***(Gordon et al 1971, Kannel et al 1986, Show et al 1987)***.

The gap in prevalence of coronary heart disease between both sexes decrease with advancing age and is not attributable to difference in life style. It seems likely that some feature of reproduction physiology are responsible for this changes.

Gordon et al 1978 in studies at Framingham indicate a prompt loss of resistance to coronary heart disease in women after attaining menopause, as compared to women of the same age who remain premenopausal. Menopause alone, more than double the risk of coronary heart disease.

Colditz et al (1987) , Lobo (1990) have shown that incidence of coronary artery disease is significantly increased, specially in those with premature menopause caused by bilateral oopherectomy or premature ovarian failure.

It has been shown that post menopausal women have higher plasma levels of cholesterol, triglycerides, very low density lipoprotein as compared to premenopausal females

(Campos et al, 1988 and Halberg et al 1987). Lower levels of high density lipoproteins were found in post menopausal period ***(Methews, et al 1989).***

The term "odd couple" has been given to LDL and HDL cholesterol which together are responsible for 90% of cholesterol in plasma. The concentration of LDL cholesterol is directly related to coronary heart disease and its elevation has been shown as a predictive risk factor ***(Gordon et al 1981) and Wilson et al, 1980.***

The majority of studies state that HDL cholesterol is a stronger predictor of risk of coronary artery disease ***(Gordon et al, 1977)*** as compared to LDL cholesterol ***(Segurdrson et al, 1975 ; Janus et al , 1980 ; Talani et al, 1981 ; Thompson et al 1981 ; Rao et al , 1983).***

LIPIDS AND LIPOPROTEINS

Increasing awareness of the relationship between plasma lipoproteins and risk factors for cardiovascular disease has lead to a renewed interest in the effect of hormone replacement therapy on cardiovascular risk. It requires an understanding of underlying mechanisms involved in the effect of replacement of hormones on serum lipids and lipoprotein.

Once the dietary fat is absorbed , the lipids are synthesized by liver and adipose tissue. The lipids are insoluble in plasma and circulate in blood stream as

macromolecular complexes. The surface layer of lipid particle consist of specific proteins called apoproteins as well as phospholipid and partially polar unesterified cholesterol. The lipid core contains nonpolar triglycerides and esterified triglycerides and cholesterol. The hydrated density of the lipoprotein particles varies with the relative ratio of its different components.

The lipoproteins can be separated by ultra centrifugation. The following system is used for classification of lipoprotein :

Very low density lipoprotein (VLDL)	.96 to 1.006
Low density lipoprotein (LDL)	1.006 to 1.063
High density lipoprotein (HDL)	1.063 to 1.21

HDL fraction → HDL2 (Major sub fraction)
HDL fraction → HDL3

Very low density lipoproteins are secreted in the liver and the endogenously synthesized triglycerides are transported into LDL. The cholesterol is transported from the liver to the peripheral cells via LDL particles. The cellular uptake of cholesterol is regulated by high affinity low density lipoprotein receptor ***(Brown et al, 1981)*** which might be influenced by estrogen. Estrogen has been found to increase the number of hepatic LDL receptors in the animal studies. This increase enhances low density lipoprotein degradation. In addition, low density lipoprotein can be broken down in scavenger cells or

macrophages of reticuloendothelial system if LDL concentration is high ***(Brown et al 1981)***. These cells can be overloaded with cholesterol and converted to foam cells.

Estrogen appears to protect against the development of cardiovascular diseases by a number of mechanisms like by beneficial changes in cholesterol levels by decreasing low density lipoprotein (LDL) cholesterol and increasing high density lipoprotein (HDL) cholesterol levels by induction of LDL receptors and destruction of hepatic lipases which degrades HDL cholesterol levels ***(Lobo RA, 1990)***. Direct effect are prevention of cholesterol deposition in vascular lesion, prevention of vasoconstrictive action of acetylcholine on artherosclerotic vessels, interference with thromboxane effect on blood vessels, increased production of prostacyclin from blood vessels, stimulation of myocardium causing a positive inotropic effect, increasing cardiac index and heart rate while decreasing systemic blood pressure and systemic vascular resistance ***(Schwartz et al, 1995)***.

1. Serum Cholesterol

Oliver and Boyd (1959) showed that there was a significant rise in serum cholesterol by oopherectomy ***Sznajderman and Oliver (1963)*** showed a significant rise in serum cholesterol in women with premature menopause compared with premenopausal women of same age group.

Arnold and Ritterband et al (1963) showed that the levels of serum cholesterol was significantly higher in oopherectomized than those of hysterectomized women.

Parcsini et al (1984) showed that there was steady rise in serum cholesterol value in women with bilateral oopherectomy within 6 months of operation.

Farrish et al (1990) showed significant increase in cholesterol level ($P<0.05$) from a mean of 3.57 mmol/l to 4.21 mmol/l.

2. Serum Triglycerides

Oliver and Boyd (1959) showed a significant elevation of serum triglycerides in bilateral oopherectomized women ***Sznajderman and Oliver (1963)*** showed that serum triglycerides were significantly raised in women with premature menopause as compared to healthy women of same age group but there was not significant increase in the level after menopause.

Punnomen and Rawaria (1976) showed that serum triglycerides level rise significantly after 1 month of bilateral oopherectomy.

Notelovitz et al (1983) showed that serum triglycerides were higher in oopherectomized women.

Farrish et al (1990) showed no significant rise in serum triglyceride level in bilateral oopherectomized women.

3. High Density lipoprotein

High density lipoprotein particle transport cholesterol from peripheral tissue including vascular endothelium to liver where it is metabolized and excreted through bile. An impairment of High density lipoprotein will accelerate excess storage of cholesterol in endothelium of arterial wall, one of the factors leading to development of atherosclerosis. Lower the level of HDL, higher the risk of atherosclecrosis.

HDL has 2 major sub fractions – HDL_2 and HDL_3.. Low level of HDL_2 are clearly related to high risk of atherosclerosis while total HDL and HDL_3 are not.

William and Kannel (1976) showed serum HDL levels are higher in women than is men.

Punnoven and Rauramo (1980) observed that HDL levels before and after one month of castration did not differ significantly.

Notelovitz et al (1981) showed that HDL levels in opherectomized women were 27% lower than others of same age.

According to ***Pausini et al (1974)*** HDL levels showed an initial decrease and later significant increase during later 3 months.

Farrish et al (1990) measured HDL subfraction to assess any change in relative amount of cholesterol. No significant change was observed in either fraction.

Low Density Lipoprotein and Very low density lipoprotein

LDL and VLDL are directly related to atherogenicity of person. Their elevated levels can be correlated with conditions favouring atherogenesis. The value of LDL are calculated from standard formula. VLDL is 20% of serum triglycerides. VLDL is supposed to carry triglycerides found in liver or possibly in the intestine to body tissue where triglycerides and fattyacids are hydrolyzed by lipoprotein lipase enzyme. Metabolites are used for energy during metabolic process and remnant left behind are taken by liver and converted to LDL. Accumulation of remnant favours atherogenesis and estrogen is reported to enhance removal of remnant.

Arnold and Ritterband et al (1963) showed that mean serum cholesterol and percentage of betalipoprotein in oopherectomized women under 50 years were higher than hysterectomized women.

Pausini et al (1984) showed initial decline, then increase in apoprotein B level – the main carrier of LDL and VLDL fraction – a significant increase upto 12.5% of premenopausal value.

Farrish et al (1990) showed a significant rise in LDL cholesterol in the 6 weeks after operation from a mean of 3.57 m-mol/l to 4.21 mmol/l.

BONE MINERAL DENSITY

Osteoporosis is characterized by absolute decrease in bone mass per unit volume of anatomical bone and microarchitectural deterioration of bone tissue with normal mineral to matrix ratio, (both mineral and matrix decreased), leading to enhanced bone fragility and increased fracture risk.

With declining ovarian function and decrease of estrogen levels, the female skeleton undergoes increasing bone remodeling and rate of bone loss increases from the perimenopausal period. The normal bone building takes place till about 30 years of age and after that, as the part of natural aging process, the break down of bone is faster than the formation. The bone mass starts declining at around 40 years and rate of loss varies between 0.25 to 1% /year , the loss being higher in trabecular (1% to 5%) than cortical bone ***(Usha Krishna, 2000)***.

Bone density , measured by bone densitometry, is expressed as grams of mineral per Cm^2.

National osteoporosis foundation recommended B.M.D. Test for

1) Post menopausal female under the age of 65 years who have one or more risk factor beside menopause.
2) All post menopausal female over 65 years regardless of risk factor
3) Post menopausal females in whom fracture has occurred.

4) For females who are on prolonged treatment of H.R.T. or results of testing would affect their decision for or against HRT.

Bone mineral density is the most sensitive and specific test for osteopenia.

It is measured by -

a) **Dual Energy X-Ray absorbimetry (DEXA)** which can be of two type

Central - for axial skeleton eg. :- Spine , hip, Whole body

Peripheral - for wrist , middle finger , forearm , heel.

b) **Single energy X-ray absorbimetry** - for forearm and heel

c) **Quantitative Computed Tomography** - for trabecular bone only eg. Vertebral body.

d) **Peripheral Quantitative tomography** - for forearm

e) **Ultrasound** - for patella, heel, ankle

f) **Quantitative** radiography.

g) **Others** - Radiogammometry
Quantitative MRI
Single and Double Photon absorbimetry
Radiographic Photodensitometry.

DUAL ENERGY X-RAY ABSORBIMETRY (DEXA)

It is the most accurate , gold standard, most widely used method now- a days. It is two dimensional and can not estimate depth or anteroposterior length of bone so cannot give true volumetric density. Results are variable measured form DEXA by different manufacturer so results of B.M.D. are compared to normal value using (T) score or (Z) score.

T - Score

Compares individual results to those in young healthy population that is matched for race and sex.

$$T = \frac{\text{BMD value (measured)- BMD (mean young)}}{\text{SD Young}}$$

Z - Score

Compares individual results to those of an age matched population that is also matched for race and sex.

$$Z = \frac{\text{BMD value (measured)- BMD (mean normal)}}{\text{SD normal}}$$

Z score is useful for people > 75 years old and in male as it is not advisable to compare them with normal healthy adults because of age related bone loss, measurement artefacts caused by osteophytes, spinal deformity extraskeletal calcification (eg. in aorta).

Interpretation

WHO has established general diagnostic categories of bone loss based on the degree of deviation from mean bone mass density (BMD) ***(Kanis JA et al, 1994)***.

Normal	: Within 1SD below normal (T Score equal to or above -1)
Osteopenia	: 1-2.5 standard deviation below normal adults (T score between - 1 and - 2.5).
Osteoporosis	: > 2.5 SD below normal, No history of fracture (T score at or below - 2.5)
Severe osteoporosis	: > 2.5SD below normal, History of non violent fractures

Advantages

Minimum radiation exposure , short scanning time, variability of repeat reading is less (1%) and ability to scan entire skeleton

Disadvantages -

It is expensive, The changes with disease progression or therapy are small in relation to the variability of measurement. Anteroposterior measurement can not be measured but this last problem can be solved by lateral densitometry.

Bone density studies should be of spine and hip. Peripheral studies (eg. : ankle, forearm, finger) are for screening only. Those patients, whose peripheral study T scores are below -1 SD should have spine and hip bone density studies.

Quantitative Computed Tomography and Quantitative MRI

They give true volumetric bone mineral density but are more expensive & less accurate. There is greater exposure to radiation.

Ultrasound

It is less accurate than DEXA or SEXA but is relatively less costly. There is no radiation hazard. So it can be used as a screening procedure.

Radiography

It is the most important method for detecting fractures in osteoporosis. It shows bone loss only when it exceeds 30% or more.

Indication

a) Decrease in height > 2.5 to 3.8 cms to rule out asymptomatic vertebral fracture.

b) Presence of kyphosis or backpain, specially in post menopausal females.

c) To rule out cause of fracture as malignancy.

Hall marks of Osteopororsis on X-Ray

a) Progressive decrease in vertebral mineral density (ground glass appearance).

b) Prominence of vertical striations and loss of horizontal trabeculations.

c) Biconcave vertebra or codfish vertebra.

d) Progressive vertebral compression with anterior wedging (reduction in anterior height) and collapse (reduction in anterior and posterior height).

Single photon absorbimetry

It has low radiation dose but is limited to peripheral skeleton and takes 10-15 minutes scan time. As the isotope has short half life, it requires regular replacement.

At present , DEXA is considered one of the best technique as its precision and accuracy are good. It has high resolution and low radiation and can measure all areas, specially the spine and proximal femur. It can be well documented and co-relates to fracture risk and scan time is less than 5 minutes ***(Usha Krishna et al 2000).***

Estrogen has positive effect on bone mass. It produces positive effect on bone mass by following actions -

Estrogen decreases osteoblastic production of interleukin-6 (IL-6), tumor necrosis factor (TNF), thereby interfering with osteoclast activation.

It helps in activation of Vit. D_3 in kidney.

It promotes positive calcium balance partly by inducing renal hydroxylase enzyme.

Bone cells express estrogen receptor α

Incidence of osteoporotic fractures in 2-3 times greater in women than men because peak bone mass is lower and there is accelerated loss after menopause ***(Melton et al, 1992)***.

Douglas P.Kiel et al, 1987 showed in a large cohort study that post menopausal use of estrogen protects against subsequent hip fracture in women.

Felson et al (1993) demonstrated that after 10 years of HRT , bone mineral content was significantly higher in HRT group compared to those who did not receive any treatment.

Daily treatment with Alendronate progressively increases the bone mass in the spine, hip and total body and reduces the incidence of vertebral fractures, progression of vertebral deformities and height loss in post menopausal women with osteoporosis (***URI A. Liberman et al, 1995***).

Post menopausal estrogen / progestin interventions (PEPI) trial, 1996 showed that HRT increases bone density in the spine , hip and produces reduction in bone turnover.

Schneider et al (1997) showed that estrogen initiated in early menopausal period and continued into late life is associated with highest bone density .

According to work of ***Delmas PD 1997***, Women receiving Raloxifene had significant increases from baseline values in bone mineral density of lumbar spine, hip , total body where as those receiving placebo had decrease in bone mineral density.

Clemett D et al 2000 , John Ston CC et al, 2000 showed that oral raloxifiene consistently increased lumbar spine, femoral neck, total hip and total body BMD relative to baseline values in post menopausal women.

Calcitonin is a synthetic hormone that inhibits bone resorption. A five year trial conducted by ***Chestnut CH (2000)*** showed that the only 200 μg dose produced significant (36%) reduction of vertebral fractures, with no reduction in hip fractures but no significant change in either bone density or bone turnover.

Black DM et al, 2000 demonstrated that Aledronate, a bisphosphonate reduces the incidence of fractures at the spine, hip and wrist by 50% in patients with osteoporosis.

ENDOMETRIAL BIOPSY

In the menopausal period, loss of effect of estrogen on uterus is that uterus becomes smaller and ratio between the body and cervix reverts to the 1:1 ratio. The uterus and cervix show gradual shrinkage due to myometrial atrophy. Estrogen withdrawal causes atrophy of endometrium and it is more marked in the functional layer than in the basal layer. The endometrium is thin i.e., only 1-3 mm in thickness with

atrophic and inactive glands. The atrophic endometrium is susceptible to infection resulting in senile endometritis and post menopausal bleeding. In rare cases, the endometrium becomes hyperplastic under the influence of extra genital estrogen (estrone) produced in the peripheral fat from epiandrostenedione.

According to ***Smith DC, 1975,*** unopposed estrogen is associated with an increased risk of endometrial cancer.

Mc Donald et al, 1977 conducted a study which indicated that the risk of developing endometrial cancer increases with duration of ERT use.

Shapiro S et al , 1985 also showed that risk may persist for more than 10 years after discontinuation of just 1 year of ERT.

Result of PEPI trial, 1995 show that women taking unopposed estrogen during the 3 year study had a significantly increased risk for endometrial hyperplasia compared with those taking estrogen plus progestins (34% versus 1%)

Boss SM, 1997 has shown in a study that Raloxifene does not induce endometrial proliferation. Statistically significant estrogenic effects were noted in 77% of estrogen treated women versus 15% of placebo treated women versus 0% of Raloxifene treated women.

Preclinical data from animal models indicates that Raloxifene does not stimulate endometrium nor does it increase uterine thickness ***(Jordon VC et al 1998)***.

Davies GC et al, 1999 have proved that Raloxifene 60 mg/d for upto 30 months is not associated with vaginal bleeding or increased endometrial thickness in post menopausal women.

Cummings SR et al, 1999 in MORE study showed that Raloxifene did not increase risk of endometrial cancer. After 40 months of followup, rate of endometrial cancer was slightly lower in these women.

Fugere P et al, 2000 proved that after a period of 24 months, the women who received ERT showed significant changes in endometrial thickness and uterine volume . In contrast,Raloxifene treated group exhibited no changes in either parameters.

In latest reports from ***MORE study, Johnson S. et al 2001*** showed that after 48 months of followup, raloxifene continues to maintain a slightly lower rate of endometrial cancer compared with placebo.

HORMONAL REPLACEMENT THERAPY

Hormone replacement therapy is available in many formulations and combination and can be given by various routes also.

Oral Preparation

HRT using estrogen alone

Cyclic estrogen progesterone preparation.

Continuous estrogen - progesterone preparation

Estrogen - androgen HRT

Non Oral Preparation

Vaginal cream and pessaries

Percutaneous gel

Transdermal patch.

Oral Preparations available are -

A. Conjugated Steroidal Estrogens

a) Estrones

Conjugated equine estrogen

Esterified estrogen

b) Estradiols

Estradiol Valerate

micronized 17 beta estradiol

c) Estriols

Estriol hemisuccinate

B. Unconjugated steroid analogue

17 Ethnyl estradiol

17 Ethnyl estradiol 3 methyl ether

17 Ethnyl estradiol 3 cyclo pentoether

C. Synthetic Estrogen Analogues

Bengestrol

Chlorotreanene

Dinestrol

Diethyestilestrol

Hexestrol

Non Oral Preparations are -

Earliest non oral routes of administration used :-

Vaginal cream

Pessaries

Subcutaneous estradiol implant

More recently

Percutaneous gel

Hormone containing vaginal rings

Most recent

Transdermal therapeutic system in the form of patch

- Only estrogen containing
- Estrogen & Progesterone.

For over three decades, estrogen replacement therapy in the form of oral conjugated equine estrogen or estradiol preparation has been widely used for relief of menopausal symptoms. But this has certain disadvantages as high doses of estrogen must be administered because of rapid metabolism to estrone and pharmacologically inactive metabolism and inactivation of estrogen within the gut wall and liver. The relatively high dosages, which are required to compensate for this, result in high peak plasma concentrations of estrone and estradiol.

Hepatic synthesis of certain protein is enhanced by high concentration of estrogens in the portal circulation, leading to increased plasma level of rennin substrate, Sex hormone binding globulin, Thyroxine binding globulin and cortisol binding globulin which leads to the development of

hypertension, gall bladder disease and thrombosis ***(Geola et al 1980 & Mandel et al, 1982)***.

Parentral administration of estrogen avoids the hepatic first pass metabolism but previously available methods have limitation that high hormone level immediately after administration (with injectable) , inadequacy of dosage adjustment (with subcutaneous pellet), inadequate systemic delivery and reduced patient acceptability (Intravaginal cream) ***(Mandel et al 1983)***.

Recently, a transdermal delivery method for estradiol administration has been developed to provide a simple approach to deliver estrogen therapy and simultaneously avoid the 1st pass metabolism through liver. It is in the form of thin adhesive patch, with drug reservoir where drug lies between occlusive backing layer and a rate controlling microporous membrane. Thus drug delivery is at constant and predictable rate.

In various clinical studies, it has been observed that beneficial effects of transdermal estradiol on plasma gonadotropin, maturation of vaginal epithelium, metabolic parameter of bone resorption and menopausal symptoms appear to be comparable to those of oral and other parentral preparation, while the undesirable effect of oral estrogen on hepatic metabolism are avoided. ***(Pedwick et al 1985), Virgil A place et al 1985 and Chalkowski et al 1986)***.

Transdermal estradiol results in increase in estradiol concentration rather than estrone concentration and it result

in plasma estradiol ; estrone ratio which more closely approximates that found in premenopausal women at early to mid follicular stage (40-100 ng/l) ***(Powers et al 1975, Selby et al 1985)***.

Non oral routes of administration of estrogen do not appear to have same magnitude of effects on lipid profile ***(Chetkowski, 1986).***

Progesterone, when properly administered, effectively prevent adenomatous hyperplasia and cause regression of pre existing adenomatous hyperplasia in the majority of the patients ***(Studd et al, 1980 ; Gambrell et al 1982 ; Gal et al 1983)***.

Progestrone reduces the concentration of estradiol E2 receptor ***(Hsuch et al, 1975)*** and increase the activity of dehydrogenase that converts estradiol to estrone, a biologically less active estrogen ***(Treng et al 1977)*** and cause endometrial cells to differentiate to a secretary state rather than proliferative state ***(Novak et al, 1979)***.

Estrogen, when combined with progesterone tend to loose some of their beneficial effects (***Knoop et al , 1986 ; Tikkanan et al, 1986)***. However , such effects are dependent on chemical structure, dose of drug and hormonal status of the recipient ***(Geola et al, 1980 , Jensen et al 1987 ; Mettssonn, 1984 ; Ottoson 1984 and La-rosa et al 1985, Bush et al, 1987).***

LDL-C is increased and HDL-C specially HDL_2 is increased due to effect of progesterone. These changes can be

correlated with increased risk of coronary heart disease in epidemiological studies ***(Wallace et al, 1987)***. However, 19-nortestosterone derivatives eg. Norethindrone, levonorgestral have a less deleterious effect on lipids and lipoproteins than C-21 progesterone derivatives such as megestral acetate, medroxy progesterone acetate.

Various studies have been done to study the effect of HRT on menopause and its effect on lipid profile.

Hallberg and Svanberg 1967 compared cholesterol phospholipid and triglyceride concentration in pre and post menopausal women. It was suggested that post menopausal women have on atherogenic risk profile , having higher plasma level of cholesterol, triglycerides VLDL, LDL than premenopausal counterparts.

No significant change in cholesterol level,significant rise in total triglyceride level, & HDL cholesterol and reduction in LDL cholesterol level was observed in women being treated with conjugated equine estrogen at a dose of 2.5 mg daily for 6 months - shown by ***Furmen et al 1967***

Utian et al 1972 compared the effect of equine estrogen with estradiol valerionate and found that their was no change in cholesterol level in women with equine estrogen but there was decrease in cholesterol level in women with estradiol valerionate.

Bengssto & Lidgnist, 1979 showed that surgically induced menopause by oopherectomy results in profound alteration of lipid balance as compared with age matched

women with intact ovaries. Significantly higher cholesterol, triglyceride and lower relative proportion of HDL were observed in oopherectomized women.

In the lipid research clinic study by ***Wahl et al 1983***, lipid profile was studied in 370 untreated post menopausal patients and 839 post menopausal patients using conjugated equine estrogen. An average decrease of 48% in serum cholesterol and 13.6% reduction in LDL cholesterol level was found in treated group as compared to that of untreated group. Also there was an average increase of 23.7% and 13% in serum triglyerides level and HDL cholesterol level in treated group. An average increase of 12.5% in LDL level was found in treated group.

Padwick et al (1985) studied the efficacy, acceptability and metabolic effect of transdermal estradiol 0.05 mg/d cyclically. The result showed that transdermal estradiol significantly increased plasma level of estradiol, estrone and urinary concentration of estradiol and produced significant improvement in menopausal symptoms and vaginal cytological findings. The drugs were well tolerated and no systemic side effects were noted.

Chetokwoski et al (1986) conducted a dose related response study in post menopausal women to compare the physiologic effects of transdermal estradiol and oral conjugated equine estrogen. The doses were 25,50, 100 and 200 ngm of transdermal estradiol/ 24 hours and .625 mg and 1.25 mg oral equine estrogen. They showed that

transdermal estradiol elicited many of the desirable actions of estrogen while avoided the deleterious pharmacological effects of oral estrogen.

Colditz GA et al (1987) analyzed prospectively a cohort of 1,21,700 U.S. Women , 30-55 years of age who were followed from 1976-1982 and suggested that in contrast to natural menopause, bilatral oopherectomy increased the risk of coronary heart disease which could be prevented by estrogen replacement therapy.

Utian WH (1987) studied that transdermal therapeutic system was both effective and well tolerated and have no effect various liver protein. Moderate bleeding occurred in patients with intact uterus which could be controlled by addition of progesterone. The incidence of endometrial hyperplasia and breast tenderness has been relatively low and minor side effects had been limited. The patients showed a preference for transdermal method of estrogen delivery.

Stanczyk FZ et al (1988) compared the efficacy of transdermal and subdermal routes of estrogen administration 20 post menopausal women were randomized to receive either two 25 mgm estradiol pellets subdermally or 1 mg estradiol transdermal patch twice weekly. Significant rise in HDL cholesterol and decrease in total cholesterol was noted at 12 weeks with pellets but only at 24 weeks with patch. Urinary calcium/creatinine ratio was reduced more consistently with the pellet than with the patch Hot flushes were eliminated in all.

Lobo RA (1990) studied that estrogen appeared to protect against development of cardiovascular disease by decreasing LDL and increasing HDL levels, by induction of LDL receptor and destruction of hepatic lipase which degrades HDL-C

Crook et al (1992) studied the comparative effects of trans dermal and oral estrogen-progesterone therapy on lipid profile at 3 and 6 month in 90 women. The regimen included transdermal estradiol 0.05 mg/d alone or combined with norethindrone acetate .025 mg/d for 14 d and oral conjugated equine estrogen .625 mg/d alone or combined with oral norgestrol 0.15 mg/dl for 12 d. Reduced serum level of total and LDL cholesterol were reported during both phases of transdermal and oral therapy with no significant effect on HDL levels during oral therapy without progesterone, whereas transdermal therapy caused 4% reduction. Oral estrogen increased serum triglycerides by 15% where as there was reduction in triglyceride level by 80%. with transdermal estrogen.

Adomi et al 1993 compared the effect on lipoprotein fraction with transdermal estrogen (0.05 mg) and oralconjugated equine estrogen (0.625 mg) in 81 post menopausal women. Both groups were combined with medroxy progesterone acetate for 12 days of the cycle. A Third group served as control. The control group experienced no observable significant changes in their lipid profile after 1 year of follow up. Both treated groups experienced significant

decrease in total LDL cholesterol, HDL cholesterol decrease in the transdermal estradiol group, rise slightly in oral estrogen group. Serum triglycerides significantly decrease in the transdermal group only.

Pong et al (1993) studied the effect of transdermal estradiol alone and when given with medroxy progesterone acetate over a 36 week period. No significant changes were observed in HDL and very low density lipoprotein cholesterol and triglycerides. Statistically significant decrease in total cholesterol were not seen until the 72nd week.

Woodruff et al (1994) studied the incidence of endometrial hyperplasia in post menopausal women taking conjugated estrogen with medroxy progesterone acetate or conjugated estrogen alone.

PEPI Trial 1995 studied the effect of estrogen or estrogen / progesterone regimens on heart disease risk factors in 875 healthy post menopausal women.

The patients were divided into following groups : -

1) Placebo
2) Conjugated equine estrogen .625 mg/dl.
3) CEE .625 mg/d plus cyclic medroxy progesterone acetate 10 mg/dl for 12 days. They found that estrogen alone or in combination with a progesterone improves lipoprotein and lower fibrinogen levels without detectable effects on post challenge insulin and blood pressure.

Unopposed estrogen is the optimal regimen for elevation of HDL- Cholesterol but the high rate of endometrial

hyperplasia restricts its use in women with an intact uterus. In women with intact uterus, CEE with cyclic MPA has the most favourable effect on HDL-C and no excess risk of endometrial hyperplasia.

Rosaw et al (1996) studied that estrogen improves serum lipid profile, carbohydrate metabolism and insulin sensitivity, prevents the formation and development of atherosclerotic plaques, reduces blood pressure and plasma fibrinogen levels and favourably affects overall cardiac features.

Four years randomized study from the university of Texas showed for 1st time an additive effect of intermittent cyclical etidronate and HRT on bone mineral density in vertebral and the hip bone ***(Jha, U.P. 1997)***.

RALOXIFENE - SELECTIVE ESTROGEN RECEPTOR MODULATOR

In order to broaden the treatment options available to post menopausal women, research efforts have been directed at the development of compounds that maintain the vasomotor, skeletal and cardiovascular benefits of estrogen replacement therapy but have little effect to no significant adverse effect on reproductive organs and the clotting process. Raloxifene is a selective estrogen receptor modulator and it belongs to BENZOTHIOPHENE class of compounds

It's biological actions are largely mediated through binding to estrogen receptors. Estrogen agonist activity is seen

on bone turnover and several cardiovascular intermediate end points such as serum lipids, lipoproteins and fibrinogen. It has no proliferative effects on uterine or mammary tissues and antagonizes estrogen effects on these tissues ***(Black LJ et al, 1983, Evans G et al, 1993 ; Sato M et al, 1994).***

Both nuclear estrogen receptors (mediating genomic effects of estrogen) and membrane estrogen receptors (mediating non genomic effects of estrogen) exist. Genomic estrogen receptors are found to have two major distinct isoforms - ERα & ERβ. β isoform is expressed more abundantly in some tissues like - bone , prostate, hippocampus where as α isoform is found primarily in breast, liver, uterus, ovary and central nervous system.

Raloxifene binds to ER α with high affinity, Similar to that of 17 β estradiol ***(Glass brook L et al, 1993)***. It also exhibited high affinity binding interactions with ER β sub type***(Gize et al, 1997)***. The unique structure allows it to bind to estrogen ligand binding pocket of estrogen receptors. Both occupy the same site but the basic side chain of Raloxifene extends out of the binding cavity and displaces one of the components of the receptor. This is a region of the ER thought to be important in some of the transcriptional activation functions of the receptors.

There are five structural domains of estrogen receptors. The AF-2 domain is a region of receptor required to activate gene sequences known to modulate estrogen activity in reproductive tissues such as the uterus and breast. The

conformation of ERα bound to estradiol is conducive to interaction with AF-2 where as conformation induced by raloxifene does not favour such an interaction.

In addition to the classic estrogen response element (ERE), multiple genomic sequences appear to exist through which ER : ligand complex may regulate transcription ***(Yang NN et al 1996)***. These may include activator protein / promotor, the retinoic acid receptor α-1 promotor, the transforming growth factor β promotor (TGFβ). Raloxifene has been shown to stimulate production of $TGF\beta_3$, a cytokine responsible for bone resorption , which substantially inhibits differentiation and resorption activity of osteoclast like cells and may account for antiresorptive properties of raloxifene on bone.

Pharmacokinetics

The drug is absorbed rapidly after oral administration. 60% of oral dose is absorbed but presystemic glucuronide conjugation is extensive. Absolute bio availability is 2% .

It can be administered without regards to meals. It is 95% bound to plasma proteins. The drug is primarily excreted in faeces and < .2% excreted unchanged in urine.

CLINICAL FINDINGS

(a) Effect on lipid profile

The estrogen agonist effects of raloxifene on lipids have been confirmed clinically. The traditional markers of cardiovascular disease risk in post menopausal women include -

Elevated levels of LDL , low levels of HDL, High triglycerides, family history of heart disease, Diabetes, hypertension, obesity, physical inactivity, smoking.

In recent years, several new markers for predicting the risk of CVD have emerged.

(i) C-Reactive Protein

Levels are higher in people with heart disease. In a 3 years study, women with the highest level of CRP had a five fold increase in the risk of developing CVD and a seven fold increase in the risk of having a heart attack or stroke ***(Ridkar PM et al, 2000.)***

(ii) Homocysteine -

Elevated levels of homocysteine can be correlated with artery damage, blood clotting, myocardial infarction, stroke and other manifestations of CVD. In women approaching menopause its levels are increased and it is thought to play a role in increased incidence of vascular disease , Cancer , osteoporosis in post menopausal women ***(Bores /GH et al, 1983, Anker G, 1995)***.

(iii) Fibrinogen

High levels of fibrinogen can restrict blood flow and lead to hardening of arteries and accumulation of plaques. Its increased levels are associated with CVD. ***(Stec JJ, 2000)***.

(iv) Lipoprotein (a)

An elevated blood concentration of lipoprotein (a) is associated with an increased risk of atherosclerosis and coronary artery disease (***Zenner G, 1986)***.

Love RR et al (1991) ; Draper MW, (1996) have conducted studies showing that raloxifene is capable of reducing LDL levels without affecting HDL or triglycerides levels significantly in post menopausal women.

Sheoman DA et al (1994) have shown that raloxifene and tamoxifen decrease the levels of lipoprotein a.

According to ***Grey AB et al, 1995.*** SERMS specially raloxifene have a greater ability to lower fibrinogen levels compared with HRT.

Delmas PD et al (1997) Blum A et al (1998) show favourable effect of raloxifene on serum concentration of total and LDL cholesterol.

Mjatovic V et al (1999) demonstrated that long term raloxifene treatment significantly lowers serum lipoprotein (a) levels in post menopausal women.

Burckhardt P, Khovidhunkit W, Agnusdei D et al, (1999) have shown the cholesterol lowering effect of raloxifene.

According to ***Vonholst T (2000) De leo V (2001),*** Raloxifene at a dose of 60 mg/d reduces serum concentration of LDL cholesterol and total cholesterol as well as cause reduction in fasting homocysteine levels.

(b) Effect on Osteoporosis

Osteoporosis is now defined as a skeletal disorder characterized by compromised bone strength, which predisposes the patient to an increased risk of fracture. The element of bone strength are bone density and bone quality, former being measured by bone densitometry. Bone quality

includes bone architecture, turnover damage accumulation (eg:- microfractures), mineralization.

Delma PD et al (1997) demonstrated that women receiving each dose of raloxifene had significant increases from baseline values in bone mineral density of lumbar spine, hip and total body where as those receiving placebo had decreases in bone mineral density.

Burckhardt P., Khovidhunkit W, Agnusdei D (1999) demonstrated that Raloxifene can be used in women free of climacteric symptoms for the prevention and treatment of post menopausal osteoporosis, alone or in combination with calcium , vitamin D., bisphosphonates and calcitonin.

Raloxifene prevents post menopausal bone loss (lumbar vertebrae, hip, radius and reduces the risk of osteoporotic fracture ***(Fontana A et al, 1999)***.

Bruce Ettinger et al (1999) demonstrated that compared with placebo group, bone mineral density increased after 36 months by 2.1% and 2.6% at the femoral neck and spine in the 60 mg raloxifene group & by 2.4% 2.7% at the femoral neck and spine in the 120 mg raloxifene group.

According to Von Holst T. (2000), there is evidence that bone mineral density is growing with treatment. In a three year study (MORE), a statistically significant decrease of lumber spine fractures was demonstrated.

(c) Effect on Endometrium -

One particular advantage of Raloxifene over HRT is its lack of proliferative effect on endometrial tissue.

Susan M. Boss, BS et al, 1997 performed double blind placebo controlled 8 week study which showed that raloxifene did not induce histopathologic evidence of endometrial stimulation in healthy post menopausal women.

Delma PD et al (1997) showed that endometrial thickness was similar in the raloxifene and placebo groups at all times during the study.

According to Burckhardt P et al, Khovidhunkit W et al (1999), SERMS can exert the known estrogen like effects on bone and lipids without exerting any action on the endometrium and the breast, a potentially ideal profile for post menopausal hormone replacement treatment. Endometrial thickness was unchanged during raloxifene therapy and no patients developed proliferative endometrium. In estrogen deficient state, raloxifene acts as an estrogen antagonist. A high estrogen milieu blunts the antagonistic effects of raloxifene on the uterus.

Endometrial neutrality of raloxifene was also demonstrated by ***Cano A, 2000.***

Fugere P et al, 2000 compared the uterine effects of raloxifene with continuous combined HRT in post menopausal women. Assessment was done by endometrial biopsy and transvaginal ultrasonography. In the raloxifene group at the end point of study, 94.4% showed normal benign post menopausal endometrium where as 78.7% of patients treated with continuous combined HRT showed normal benign post menopausal endometrium. Mean endometrial thickness was

unchanged from baseline with raloxifene and was increased by 5 mm with continuous combined HRT.

(d) Protective Effect on breast Cancer -

Raloxifene has antiproliferative effect on human breast cancer cells and inhibits mammary carcinogenesis in animal models of breast cancer.

Freedman M et al, 2001 assessed changes in mammo graphic density in healthy post menopausal women in osteoporosis prevention trial.The study showed that the women who received placebo showed a significant decrease in breast density as did the women who received raloxifene. The women, who received estrogen on the other hand, experienced a non significant increase in breast density.

MORE trial, 2001 in post menopausal women with osteoporosis showed that raloxifene reduced the risk of newly diagnosed invasive breast cancer by 72% in these women. The risk of estrogen receptor positive invasive breast cancer was reduced by 84%.

Adverse Effects

Raloxifene at doses upto 150 mg/day has been well tolerated in clinical trials in post menopausal women. No clinically important changes in hematological, renal or hepatic laboratory variables were observed

The only adverse effect possibly related to Raloxifene was vasodilatation (hot flushes), most common in the raloxifene Hcl 600 mg group, according to ***Draper MW, et la 1996***.

According to ***Delmas PD et al, 1997*** the proportion of women receiving raloxifene who reported hot flushes or vaginal bleeding was not different from that of women receiving placebo.

Burckhardt P., 1999 has shown that as in hormone replacement therapy, thromboembolism and leg cramps occur more frequently.

Agnusdei D. et al, Davis GC et al, 1999 have shown that hot flushes were the most frequently observed undesirable effects at frequency slightly higher in the raloxifene group (25%) than in the placebo group (18%).This undesirable effect was of the low intensity and generally occurred during 1st 6 months of treatment. It did not cause a higher drop out rate.

According to ***Weerapan Khovidhunkit, et al, 1999*** , incidence of breast tenderness and vaginal bleeding with raloxifene was similar to that seen with placebo but significantly less than that produced by estrogen therapy. The most serious side effect associated is a three fold increase in the risk for venous thromboembolism, an increase similar to that seen with estrogen therapy.

Material & Methods

MATERIAL AND METHOD

The present study was carried out in the department of Obstetrics & gynaecology, M.L.B. Medical College, Jhansi (U.P.) The patients were selected from gynaecology OPD and ward.

Total 50 postmenopausal females with amenorrhoea of more than 6 months duration, were selected for the study.

1. SOURCE :

Patients are selected from -		OPD
	-	Gynaecology ward
Natural menopause	-	Age above 40 years with more than 6 months amenorrhoea
Artificial menopause	-	Hysterectomy with Bilateral oopherectomy.

2. CRITERIA :

(a) Presenting Complaints

Hot flushes, night sweats, insomnia, dry vagina, dyspareunia, palpitations, burning micturition, vaginal discharge, frequency and urgency of urine, bone pains, spontaneous fracture.

(b) History of the patient

Name, age, parity, caste, socio-economic status, place of residence, occupation.

Age of menopause

Duration of menopause

Type of menopause whether natural or artificial

Menstrual history.

Obstetrical history.

Personal History. - H/o smoking , Alcohol , H/o past surgery.

Past history - Tuberculosis, Hypertension , Ischemic heart disease diabetes mellitus, malignancy.

Drug History.

(c) Clinical Examination

General Examination - General condition, weight , blood pressure, pulse rate, respiratory rate, body temperature, pallor, cyanosis, jaundice, oedema, lymphadenopathy

Chest Examination

Cardiovascular System -

Any evidence of coronary artery disease, hypertension and thromboembolic phenomenon.

Central Nervous System -

Irritability, loss of memory, any psychological symptom.

Per Abdomen Examination

Breast Examination

Any lump, discharge, fixity, tenderness

Per Speculum and per Vaginum Examination

Condition of vulva

Condition of vagina and any vaginal secretions

Any pathology of cervix.

Size and position of uterus and adnexa.

(C) Investigations

Hb%, TLC, DLC, ESR

Urine, Routine & Microscopic

Blood Sugar,

Blood Urea

Electrocardiogram

Lipid Profile

Serum total cholesterol (STC)

Serum Triglycerides (STG)

High Density Lipoproteins (HDL)

Low Density Lipoproteins (LDL)

Method of Collection of Blood sample for Lipid profile Estimations

5 ml of blood was with drawn from antecubital vein of the female in recumbent posture with all aseptic precautions

- After 12 - 14 hours fasting.
- Without venous stasis

After that withdrawn blood was allowed to settle down for half an hour, then centrifuged and serum was preserved with standard precaution.

Period of Collection of Sample

1. Basal Sample i.e. before starting the therapy.
2. After 3 months of Therapy
3. After 6 months of therapy.

Estimation of Lipid Factors

Various lipid factors, serum total cholesterol (STC), serum Triglycerides (STG) High density lipoprotein (HDL) were estimated by diagnostic kits, while low density lipoproteins (LDL) and very low density Lipoproteins were derived from the values of above mentioned lipids by standard formulae.

1. Estimation of Serum Total Cholesterol

Serum total cholesterol (STC) was estirnated by a commercial kit supplied by Ethnor. The basic principle is that cholesterol reacts with kit solution of Ferric perchlorate, Ethyl Acetate and sulphuric Acid and gives lavender colour complex which is measured calorimetrically.

2. Estimation of serum Triglycerides (STG)

Serum Triglycerides were estimated by acetyl acetone method. The principle behind this is that triglycerides are determined by measured glycerol. After its liberation from fatty acids by saponification glycerol is oxidized by sodium

metaperiodate to formaldehyde which is directly proportional to the amount of triglycerides.

3. Estimation of High Density Lipoproteins (HDL)

HDL was estimated by utilizing commercial kit supplied by Ethnor. The basic principle is that HDL cholesterol fraction is separated by using a precipitating reagent. The precipitants contain chylomicrons, VLDL, LDL which are removed by centrifugation. The supernatants contain HDL-C which is estimated by HDL-C colour reagent which gives purple colour complex. Intensity of colour developed is proportional to the concentration of HDL cholesterol in the specimen under test.

4. Estimation of Very low denstiy lipoproteins (VLDL)

VLDL was estimated by formula given by ***Fried wald et al (1972)*** this formula is valid upto STG values to less than 400 mg%.

$$VLDL\ (mg/dl) = STG/5.$$

5. Estimation of low denstiy lipoproteins (LDL)

LDL was estimated by using ***fredrickson DA (1972) formula***

$$LDL\ (mg/dl) = STC - (STG/5 + HDL)$$

$$= STC - (VLDL + HDL)$$

ENDOMETRIAL BIOPSY

Preparation of the patient - A written consent of the patient was taken. She was asked to evacuate her bladder.

Cases, who were unable to evacuate bladder, were catheterized by plain catheter. Sedation was given to the patient in the form of Inj. Pentazocine 30 mg. with Inj. Promethazine 20 mg. For taking endometrial biopsy, patient was made to lie down in lithotomy position.

Technique -

The vulva was painted with antiseptic solution followed by cleaning of vagina with antiseptic solution. The part was draped by a sterile cut sheet. The vaginal examination was done to know the size of the uterus, whether anteverted or retroverted position of the cervix. The posterior vaginal wall was retracted by sim's speculum. The anterior vaginal wall was retracted by anterior vaginal wall retractor. The volsellum was used to hold the anterior lip of cervix. A uterine sound was passed to know the length of the uterine cavity and patency of cervical os determined. If dilatation of cervical os was required, it was done with gradually increasing size of Hegar's dilator, so that endometrial curette can be introduced. An endometrial biopsy curette was introduced and curettage of endometrial cavity done. The curettings obtained were placed in a bottle containing formalin.

Interpretation of Histology Slides (Anderson' pathology volume 2, Tenth edition)

a) *Early Proliferative* - During early proliferative phase (days 5-7), endometrial glands are sparse, straight , narrow

(tubular). They are lined by columnar epithelium with few mitoses and little or none pseudostratification. The stroma is dense.

b) *Mid proliferative phase* - During mid proliferative phase (days 8-10), the glandular epithelium shows increasing pseudostratification and more mitoses as compared to early proliferative phase. The stroma is loosened by oedema.

c) *Late proliferative* - There is spiralling of glands and vessels. Glands are cystic, tortuous. The stroma is dense, diffuse stratification and mitoses are abundant.

d) Early secretory phase - A subnuclear vacuole appears and this gradually moves towards periphery of cell and then extended into lumen of the gland. The stroma is loose, blood vessels are dilated.

e) *Late Secretory Phase* - The gands are filled with secretion. The luminal borders are frayed. The stroma is loose and shows decidual reaction. There is infiltration of leucocytes.

f) *Endometrial Hyperplasias* -

Silverberg classified the hyperplasias into four categories which are in the increasing order of severity - simple, cystic, adenomatous and atypical.

i) ***Simple hyperplasia*** -

There is increased thickness of endometrium , increased crowding of glands and evidence of estrogenic activity. The glands of simple hyperplasia are generally small, round and regular and fail to show cellular atypia. The glands are generally crowded and morphology of proliferative phase is universally encountered.

ii) ***Cystic Hyperplasia*** -

Co-existence of large cystically dilated glands and small, round glands in a voluminous endometrial glands. Glands are round in shape, neither any irregularity nor cellular atypia is encountered. There is stratified epithelium with proliferative activity seen in cystic Hyperplasia.

iii) Adenomatous Hyperplasia

Above findings plus irregularity and abnormal shape of hyperplastic endometrial glands. A typical finding is the presence of small buds projecting from larger, often cystically dilated glands. Amount of endometrium obtained at curettage is more voluminous in adenomatous hyperplasia, then in the less severe forms.

iv) Endometrial Carcinoma

Endometrial adenocarcinoma are usually glandular in pattern with a smaller percentage being papillary. Increasing anaplasia of tumour is manifested by a tendency to grow in solid nests or sheets with less formation of glands or papillae.

Grade I adenocarcinoma are composed entirely of tumour growing in glandular or papillary. Pattern. Grade II tumors have an intermediate pattern of growth, there is moderate differentiation of tumour cells manifesting predominantly glandular elements but with a mixture of solid growth pattern. Grade III tumours comprise of poorly differentiated lesions growing predominantly in solid sheet of cells.

Carcinoma in situ in used to denote a small focus of tumour in an otherwise benign curettings. There is tufting & infolding into glands lumen. The cells in each instance are tall, pale and even with hyperplasia. There may be some evidence of secretory activity.

Estrogenicity Scoring system - Step 1 : Score Individual biopsy specimens according to eight morphologic features

Morphologic feature	***None (0)***	***Limited (1)***	***Higher (2)***
Glandular effects			
Shape	Small, tubular, straight	Open, straight	Open/cystic, tortuous
Nuclear/cytoplasmic ratio	Very high (>75%)	Moderate (75%-50%)	Low (<50%)
Pseudostratification	None	Limited	Diffuse
Mitoses	None	Rare	Scattered to many
Stromal effects			
Density	Compact or fibrous	Loosely cellular	Edematous
Mitoses	None	Rare	Few to many
Other effects			
Metaplasias	None	Rare	Few to many
Tissue volume	Disrupted or scant intact	Moderate- Much being intact	Abundant Intact

Estrogenicity scoring system - Step 2 : Total morphologic feature scores and assign estrogen effect grade

Total score	*Estrogen effect grade*	*Description*
0-3	0	Typical post menopausal endometrium with little or no estrogenic effect
4-6	1	Definite but limited estrogenic effect
7-10	2	Significant estrogenic effect
>10	3	Marked estrogenic effect

BONE MINERAL DENSITY (BMD)

Osteoporosis is a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue with consequent increase in fragility and susceptibility to fracture.

Bone mass is measured by bone densitometery. In this study, it was measured by peripheral dual energy X-ray absorbimetry (P-DEXA) and region involved was wrist of left hand. It was done before starting the therapy and after 6 months of treatment.

Interpretation of BMD Results

WHO has established general diagnostic categories of bone loss based on the degree of deviations from mean bone mass density.

Normal	: Within 1SD below normal (T Score equal to or above -1)
Osteopenia	: 1-2.5 standard deviation below normal adults (T score between - 1 and - 2.5).
Osteoporosis	: > 2.5 SD below normal, No history of fracture (T score at or below - 2.5)
Severe osteopororsis :	> 2.5SD below normal, History of non violent fractures

' T ' Score - is generally used to relate the results to normal values found in healthy young population, that is matched for race & gender.

' Z ' Score - compared the individual results to those of an age matched popualtion that is also matched for race & sex.

Exclusion of Patients :

Following patients were excluded from study such as -

- Patients with undiagnosed vaginal bleeding, genital neoplasm, carcinoma breast or history of carcinoma breast in family.
- Patients who had any major complications in post operative period.
- Patients with cardiovascular disease, hypertension, Diabetes mellitus. History of jaundice and thrombo embolic phenomenon were not included in this study.

Selection of Patients :

Selected from the patients attending the OPD of department of obstetrics & gynaecology , M.L.B. Medical College, Jhansi.

A separate record of such patients was kept in register. This procedure was adopted till the number of post menopausal patients attained the number fifty. Datewise serial no was given to the cases. These fifty patients were divided into two groups of twenty five each.

The selection of cases for the various drugs was made on the basis of systemic random sampling.

Mode of Administration of Drugs :

Group I - Prescribed Tab, Premarin .625 mg/day.

Group 2 - Prescribed Tab. Ralista 60mg/d OD Both the groups were given calcium tablets along with the other drug.

Clinical examination was done at 0,3 and 6 months lipid profile was done at 0, 3, 6 months whereas bone mineral density at 0 and 6 months. Endometrial bipopsy was done in those with intact uterus at 0 and 6 months.

Enquiry was made upon -

a) Control of symptoms

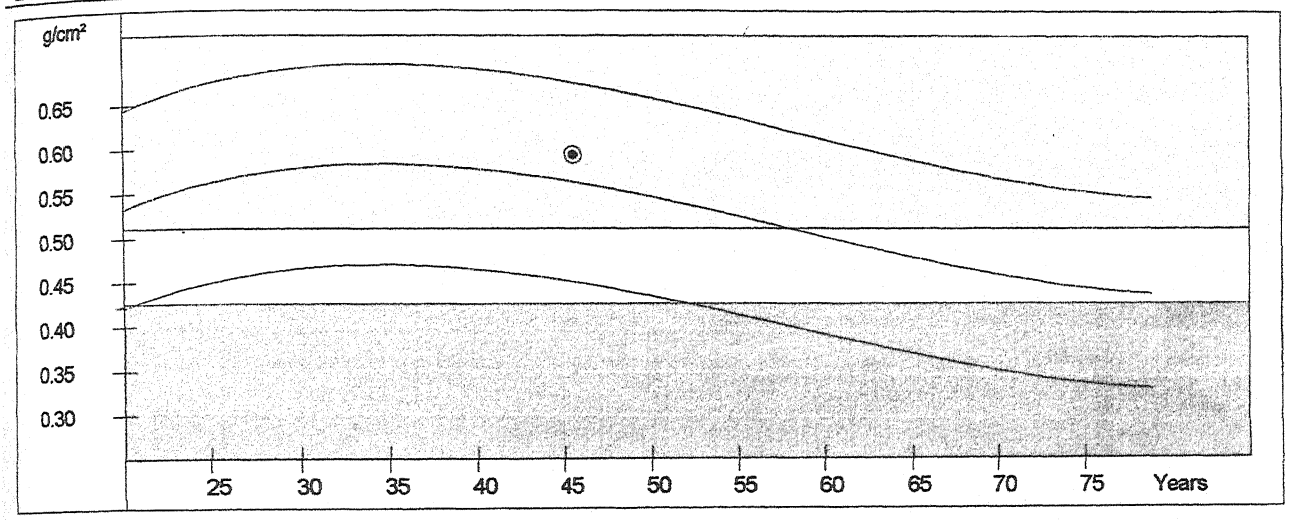
b) Any complaints like hot flushes, vaginal bleeding, breast pain or leg cramps.

c) weight gain

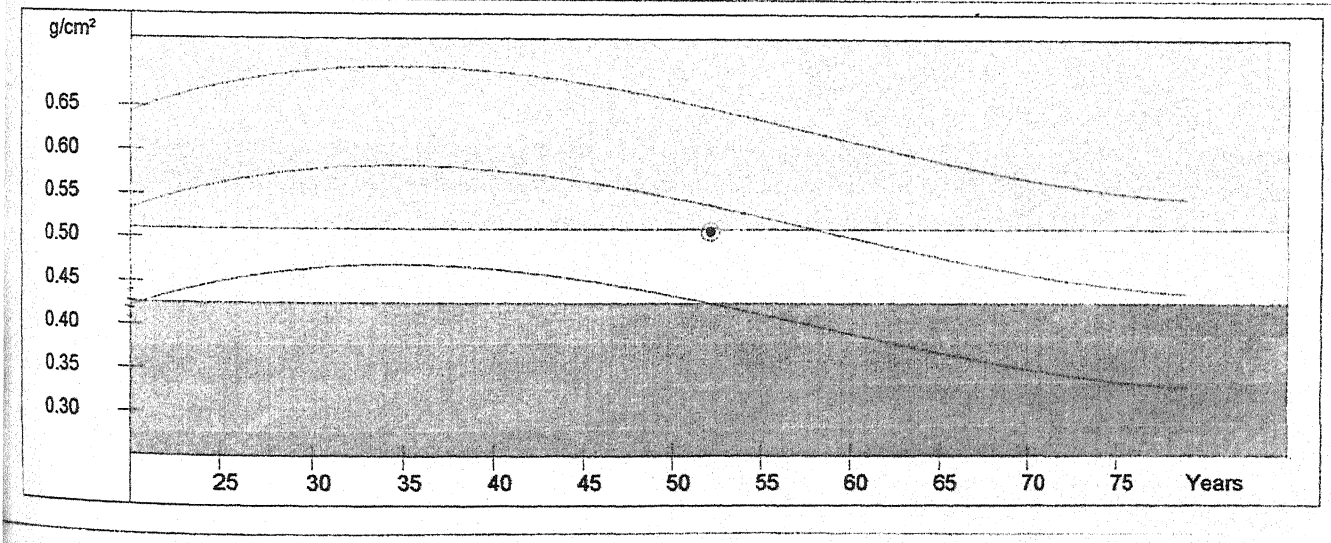
d) Blood pressure

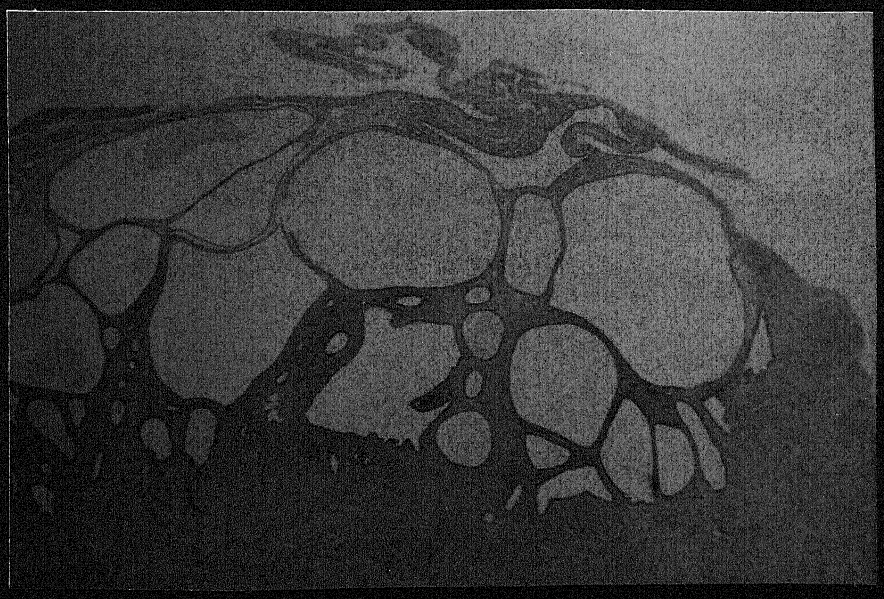
e) Breast Examination.

BMD reference graph: OF POST MENOPAUSAL WOMAN SHOWING NORMAL BMD



BMD reference graph: OF POST MENOPAUSAL WOMAN SHOWING OSTEOPENIA





POST MENOPAUSAL ATROPHY OF ENDOMETRIUM

SIMPLE ENDOMETRIAL HYPERPLASIA

Observations

OBSERVATION

In the present study a total of 50 postmenopausal subjects with either natural or surgical menopause having menopausal symptoms enrolled for observing the effect of replacement therapy on symptomatic relief, lipid profile and bone mineral density and endometrial biopsy into two groups.

Group - I 25 postmenopausal women with natural/ surgical menopause enrolled in this group. They were given oral conjugated equine estrogen (Premarin) 0.625 mg/day. The duration of therapy was 6 months.

Group - II 25 postmenopausal women with natural / surgical menopause enrolled in this group. They were given oral Raloxifene 60mg/day along with calcium (oral) 500mg/day. The duration of therapy was 6 months.

Participants in all the two groups were matched for general characteristics like age, age at menopause, duration of menopause, parity, socio-economic status.

Following observations were made

Table - I

Distribution of cases according to age in both the groups

Age	Group I		Group II		Total	
	n = 25	%	n = 25	%	N = 50	%
36-40 Yrs.	8	32	6	24	14	28
41-45 Yrs.	11	44	8	32	19	38
46-50 Yrs	6	24	11	44	17	34
51-55 Yrs.	Nil	-	Nil	-	Nil	-
Total	***25***	***100***	***25***	***100***	***50***	***100***

Mean age of group I subjects is 42.56 + 3.16 years

Mean age of group II subjects is 44.2 + 3.82 years

		Significant
t = 1.67	p > .05	NS

As shown in table I, majority of subjects in group I 11 (44%) were between 41-45 years. In group II, majority of subjects 11(44%) were between 46-50 years.

There was no significant difference statistically in the mean age of subjects in both the groups.

Table - II
Distribution of cases according to age of onset of Menopause in both the groups

Age	Group I		Group II		Total	
	$n_1 = 25$	%	$n_2 = 25$	%	N = 50	%
36-40 Yrs.	12	48	11	44	23	46
41-45 Yrs.	11	44	9	36	20	40
46-50 Yrs	2	8	5	20	7	14
51-55 Yrs.	Nil	-	Nil	-	Nil	-
Total	***25***	***100***	***25***	***100***	***50***	***100***

- Mean age of onset of menopause in group I subject is

  40.2 + 3.2 years

- Mean age of onset of menopause group II subject is

  41.7 + 1.7 years

$t = 0.24$ $p > 0.05$ Significant / NS

As shown in table II majority of subjects 12 (48%) in group I attained menopause between 36-40 years while in group II, 11 (44%) subjects attained menopause between 36 - 40 years. There was no significant difference statistically in the age of onset of menopause in both the groups.

Distribution of Cases according to age of onset of Menopause in both the groups

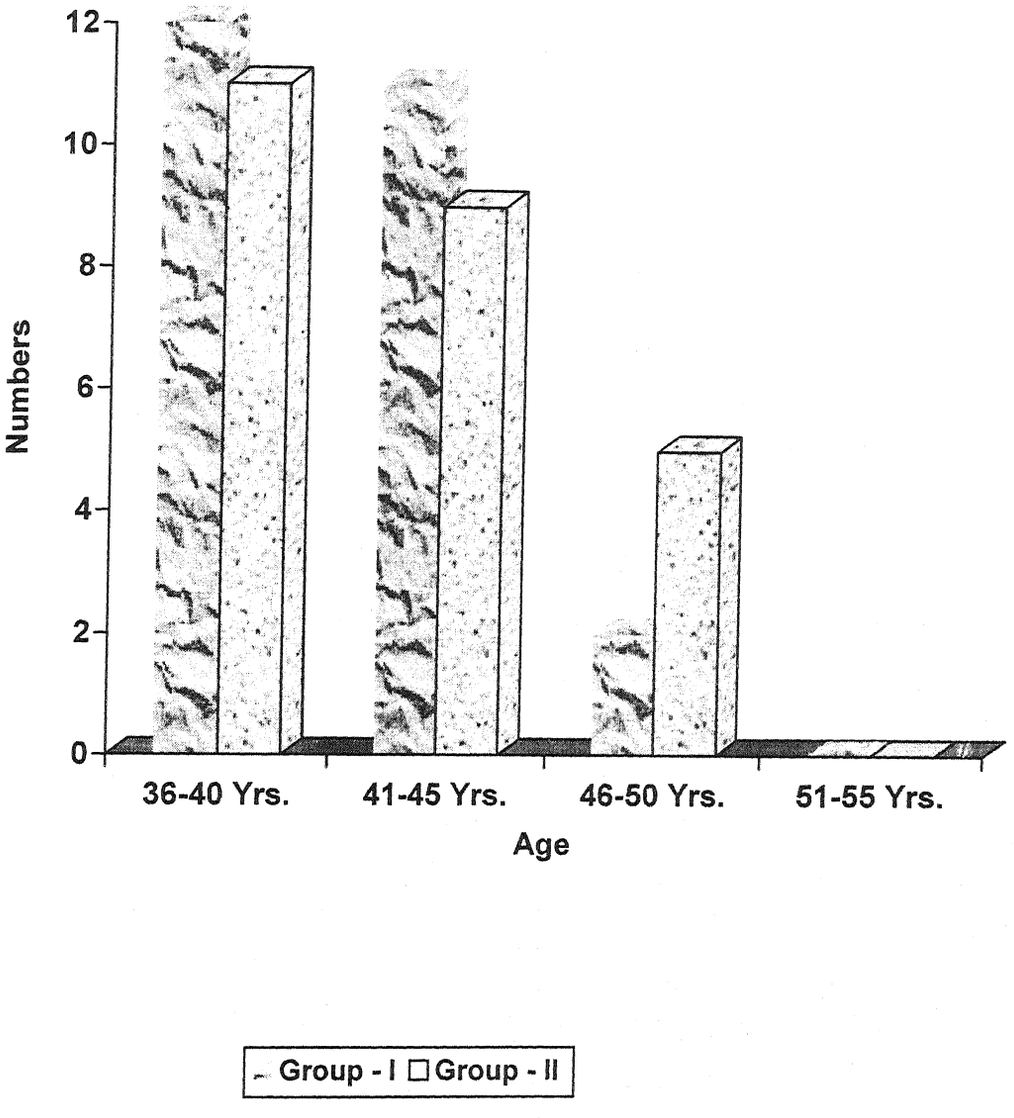


Table - III

Distribution of cases according to duration of menopause in both groups

Duration of Menopause	Group I		Group II		Total	
	$n_1 = 25$	%	$n_2 = 25$	%	N = 50	%
upto 1 year	15	60	9	36	24	48
> 1-3 years	8	32	10	40	18	36
> 3-5 years	1	4	5	20	6	12
> 5 years	1	4	1	4	2	4
Total	***25***	***100***	***25***	***100***	***50***	***100***

Mean $\pm$ S.D. in group I = 1.36 years $\pm$ 1.26

Mean $\pm$ S.D. in group II = 2.12 years $\pm$ 0.96

As shown in table III majority of subjects 15 (60%) in group I and 9 (36%) in group II had duration of menopause upto 1 years.

While 8 (32%) subjects in group I and 10 (40%) subjects in group II had duration of menopause 1-3 years.

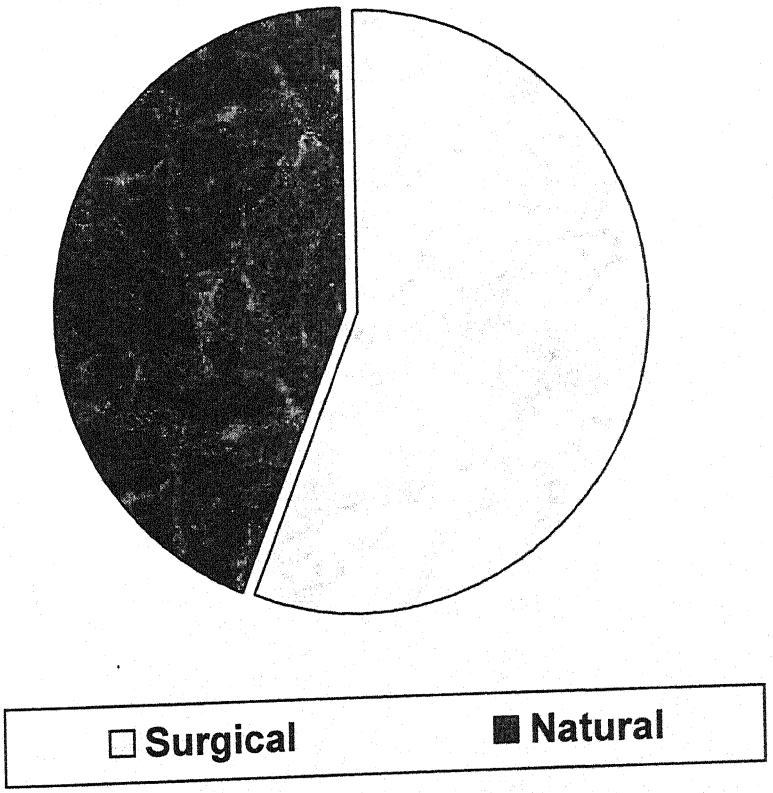
Table - IV

Distribution of cases according to type of menopause in both groups

Type of Menopause	Group I		Group II		Total	
	$n_1 = 25$	%	$n_2 = 25$	%	N = 50	%
Surgical	14	56	13	52	25	50
Natural	11	44	12	48	25	50
Total	***25***	***100***	***25***	***100***	***50***	***100***

As shown by above table majority of subjects in group I had surgical menopause (56%) whereas in group II, majority of subjects had surgical menopause (52%).

Distribution of Cases According to type of menopause in group - I



Distribution of Cases According to type of menopause in group - II

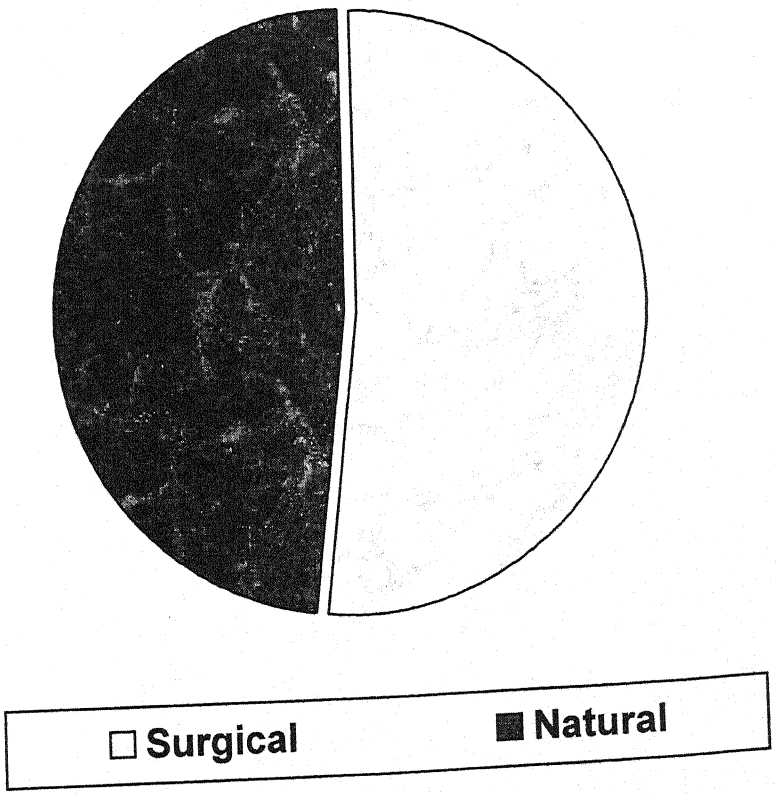


Table - V

Distribution of cases according to age of menopause in both i.e. natural & Surgical menopause groups

Age	Group I		Group II		Total	
	$n_1 = 25$	%	$n_2 = 25$	%	N = 50	%
36-40 Yrs.	2	4	20	40	22	44
41-45 Yrs.	17	34	6	12	23	46
46-50 Yrs	4	8	1	2	5	10
51-55 Yrs.	Nil	-	Nil	-	Nil	-
Total	***23***	***46***	***27***	***54***	***50***	***100***

Mean age of natural menopause = 43.32 $\pm$ 2.05 year
Mean age of Surgical menopause = 39.32 $\pm$ 1.21 year

Distribution of Cases according to age of Menopause in both i.e. natural & Surgical Menopause groups

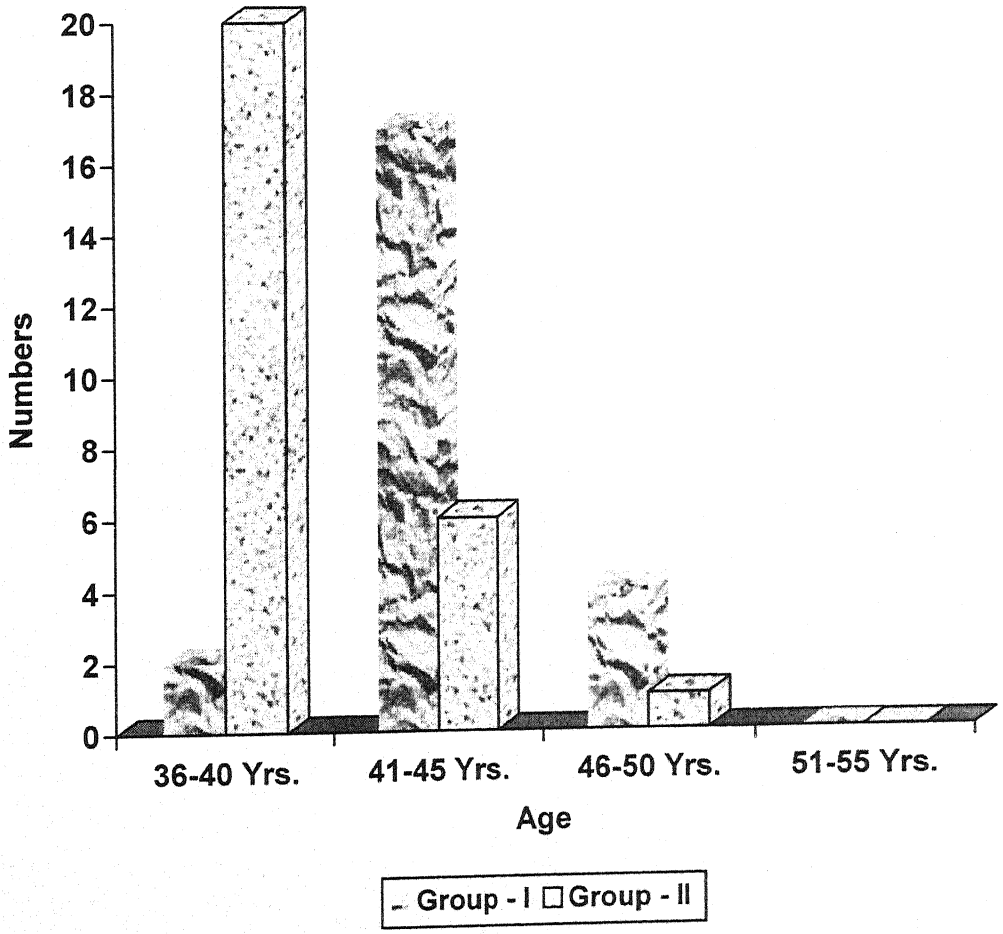


Table - VI

Distribution of cases according to Parity in both group

Parity	Group I		Group II	
	$n_1 = 25$	%	$n_2 = 25$	%
P_1	Nil	-	Nil	-
P_2	2	8	2	8
P_3	8	32	9	36
P_4	15	60	14	56
	Nil	Nil	Nil	Nil
Total N=50	25	100%	25	100%

As seen by above table, there were no nullipara or primipara. The maximum number of patients were of parity 3 in both the groups. 60% in group I and 56% in group II.

Table - VII

Distribution of cases according to socio economic status (according to Kuppuswamy Socio-economic scale)

SES	Group I		Group II		Total	
	$n_1 = 25$	%	$n_2 = 25$	%	N = 50	%
Upper	Nil	-	Nil	-	Nil	-
Upper- Middle	9	36	5	20	14	28
Lower- Middle	12	48	16	64	28	56
Lower	4	16	4	16	8	16
Total	***25***	***100***	***25***	***100***	***50***	***100***

According to the above table, 12 (48%) subjects in group I and 16 (64%) subjects in group II belong to lower middle class.

Table - VIII

Distribution of cases according to clinical symptom during study in Both groups.

Symptoms	Group I		Group II	
	n	%	n	%
Hot Flushes	20	80	18	72
Sweating	13	52	9	36
Palpitations	16	64	13	52
Dizziness	3	12	3	12
Paraesthesia	3	12	2	8
Nervousness	7	28	5	20
Fatigue	4	16	4	16
Depressive symptoms	16	64	13	52
headache	4	16	3	12
Insomnia	3	12	11	44
Dyspareunia	2	8	6	24
Backache	14	56	11	44
Joint pain	12	48	8	32

The common complaint of subjects in both the groups were hot flushes (80% in gp. I , 72% in gp II), palpitation (64% in group I, 52% in group II), Depressive symptoms (64% in group I & 52% in group II), Backache (56% in group I and 44% in group II), Sweating (52% in group I), Insomnia (44% in group II), 8% in group I & 24% in group II presented with dyspareunia.

Table - IX

Distribution of subjects according to improvement in symptoms in both groups after 3 months and 6 months of therapy.

Improvement in Symptoms	Group I				Group II			
	After 3 months		After 6 months		After 3 months		After 6 months	
	n	%	n	%	n	%	n	%
No Improvement	2	8	1	4	15	60	8	32
Improvement to some extent	8	32	4	16	10	40	17	68
Significant Improvement	15	60	20	80	Nil	-	Nil	-
Total N=50	25	100	25	100	25	100	25	100

No improvement → patient not relieved of symptoms at all

Improvement to some extent → upto 50% relief in symptoms

Significant Improvement → > 50% relief in symptoms

Table IX shows that significant improvement of symptoms seen in 60% in group I and None of the patients in group II after 3 months of therapy. While 80% in group I and none of the patients in group II after 6 months of Therapy.

Improvement to some extent has been observed in 32% of group I and 40% in group II subjects after 3 months of therapy while 16% in group I and 68% in group II after 6 months of therapy.

No improvement in symptoms was observed in 8% in group I and 60% in group II after 3 months while 4% in group I and 32% in group II after 6 months of therapy

Table - X

Showing Comparison of HDL levels in both the groups at basal, 3 months and 6 months of therapy

Duration of Therapy	Group I (mean ± SD) (mg/dl)	Group II (mean ± SD) (mg/dl)
basal (a)	40.36 ± 6.00	42.08 ± 5.42
At 3 months (b)	40.76 ± 5.88	42.26 ± 5.44
At 6 months (c)	44.12 ± 3.65	42.60 ± 5.56

Applying paired 't' Test to the values shown in the master chart, following results were obtained :-

In Group I

At basal V/s 3 months
a : b , t = 3.47 p < .05 Significant

At 3 months V/s 6 months
b : c , t = 3.80 p < .05 Significant

In Group II

At basal V/s 3 months
a : b , t = 2.00 p > .05 Not Significant

At 3 months V/s 6 months
b : c , t = 2.03 p > .05 Not Significant

On comparing HDL levels in Table X, it was found that there was significant increase in HDL levels in Group I whereas there was no significant increase in HDL levels in Group II.

There was 9.31% increase in the level of HDL in group I was statistically significant.

Table - XI

Showing Comparison of LDL levels in both the groups at basal, 3 months and 6 months

Duration of Therapy	Group I (mean ± SD) (mg/dl)	Group II (mean ± SD) (mg/dl)
basal (a)	147.64 ± 10.39	141.36 ± 17.42
At 3 months (b)	139.24 ± 9.05	131.04 ± 18.51
At 6 months (c)	136.20 ± 8.46	130.12 ± 17.52

Applying paired 't' Test to the values shown in the master chart, following results were obtained :-

In Group I

At basal V/s 3 months
a : b , t = 6.37 p < .01 highly Significant

At 3 months V/s 6 months
b : c , t = 9.36 p < .01 highly Significant

In Group II

At basal V/s 3 months
a : b , t = 4.8 p < .01 highly significant

At 3 months V/s 6 months
b : c , t = 5.08 p < .01 highly Significant

On comparing LDL levels in Table XI, it was found that there was significant decrease in LDL levels in both the groups.

This decrease was significant in both the groups after 3 months and 6 months. There was 7.72% and 7.95% decrease in group I and II respectively after 6 months of therapy.

Table - XII

Showing Comparison of Serum total cholesterol levels in both the groups at basal, 3 months and 6 months

Duration of Therapy	Group I (mean ± SD) (mg/dl)	Group II (mean ± SD) (mg/dl)
basal (a)	220.96 ± 24.80	233.84 ± 17.06
At 3 months (b)	206.80 ± 25.68	218.12 ± 17.19
At 6 months (c)	204.60 ± 24.92	216.20 ± 16.31

Applying paired 't' Test to the values shown in the master chart, following results were obtained :-

In Group I

At basal V/s 3 months
a : b, t = 6.17 p < .01 highly Significant

At 3 months V/s 6 months
b : c , t = 5.133 p < .01 highly Significant

In Group II

At basal V/s 3 months
a : b , t = 7.48 p < .01 highly significant

At 3 months V/s 6 months
b : c, t = 3.13 p < .01 highly Significant

On comparing STC levels in Table XII, it was found that there was significant decrease in STC in both the groups. There was 7.96% and 7.54% decrease in group I and II respectively after 6 months of therapy.

Table - XIII

Showing Comparison of STG levels in both the groups at basal, 3 months and 6 months

Duration of Therapy	Group I (mean ± SD) (mg/dl)	Group II (mean ± SD) (mg/dl)
basal (a)	165.76 ± 15.73	177.60 ± 13.253
At 3 months (b)	160.48 ± 24.01	176.52 ± 16.939
At 6 months (c)	160.60 ± 25.44	177.68 ± 16.82

Applying paired 't' Test to the values shown in the master chart, following results were obtained :-

In Group I

At basal V/s 3 months
a : b , t = 2.19 p < .05 Significant

At 3 months V/s 6 months
b : c , t = 3.06 p < .01 highly Significant

In Group II

At basal V/s 3 months
a : b , t = .40 p > .05 Not significant

At 3 months V/s 6 months
b : c , t = 1.8 p > .05 Not Significant

On comparing STG levels in Table XIII, it was found that there was decrease in STG levels in group I and this decrease was statistically significant after 3 months and 6 months respectively. There was 3.11% decrease after 6 months of therapy.

There was no significant change observed in STG levels in Group II after 3 and 6 months of therapy.

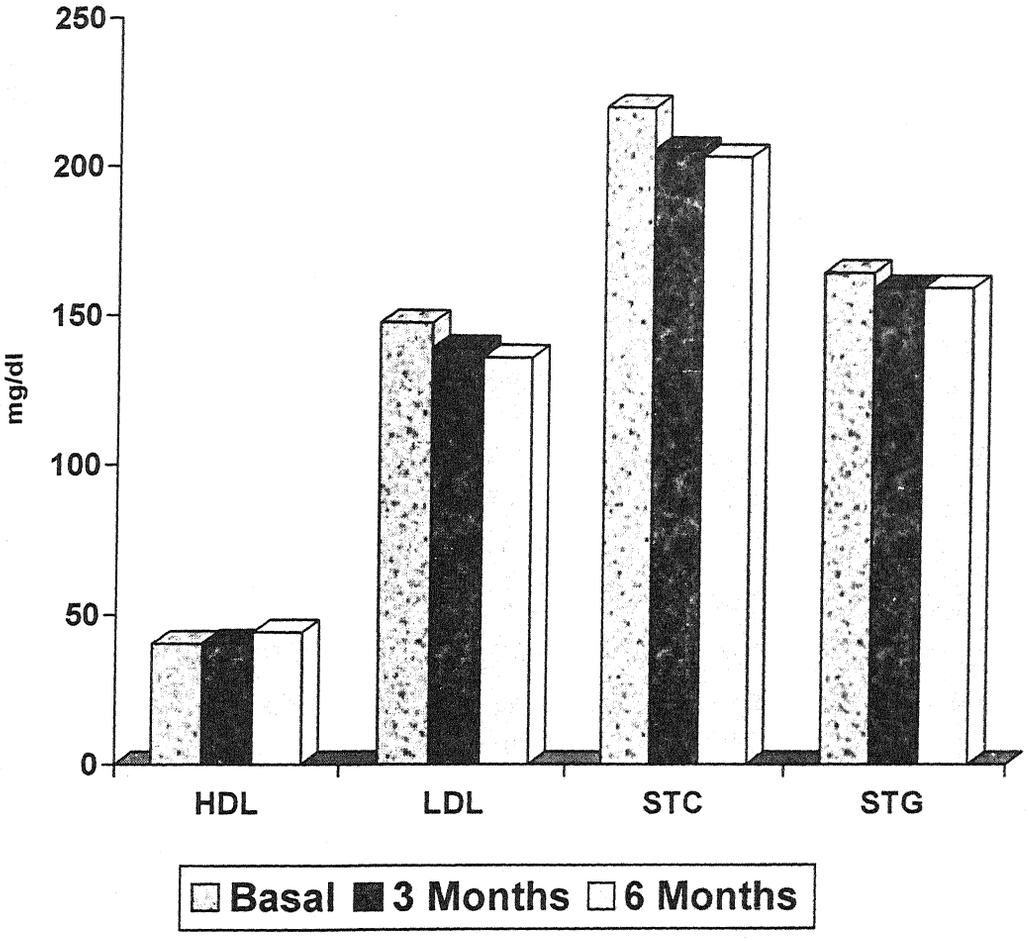
Table - XIV

Effect of Premarin and Raloxifene on serum lipids after 3 months and 6 months of Therapy (mean ± S.D., mg/dl) and statistical significance

Serum Lipids		Basal (Mean + SD) mg/dl	3 months (Mean + SD) mg/dl	6 months (Mean + SD) mg/dl	Statistical Significance Basal	3 months	6 month
HDL	Group I	40.36±6.00	40.76±5.88	44.12±3.65	1.075 p>.05	0.95 p>.05	1.15 p>.05
	Group II	42.08±5.42	42.26±5.44	42.60±5.56			
LDL	Group I	147.64±10.32	139.24±9.05	136.20±8.46	1.78 p>.05	2.00 p>.05	1.58 p>.05
	Group II	141.36±17.42	131.04±18.51	130.12±17.52			
STC	Group I	220.96±24.80	206.80±25.68	204.60±24.92	- 2.16 p>.05	- 1.85 p>.05	- 1.97 p>.05
	Group II	233.84±17.06	218.12+17.19	216.20±16.31			
STG	Group I	165.76±15.73	160.48±24.01	160.60±25.44	- .29 p>.05	- 2.75 p>.05	- 2.83 p>.05
	Group II	177.60±13.25	176.52±16.93	177.68±16.82			

Result obtained by applying paired 't' test to values shown in master chart show that there was no statistically significant difference in HDL-C, LDL-C, STC, STG level in both the groups

Mean Serum Lipid Lipoprotein Basal and after 3 months & 6 months of therapy in Group - I



Mean Serum Lipid Lipoprotein Basal and after 3 months & 6 months of therapy in Group - II

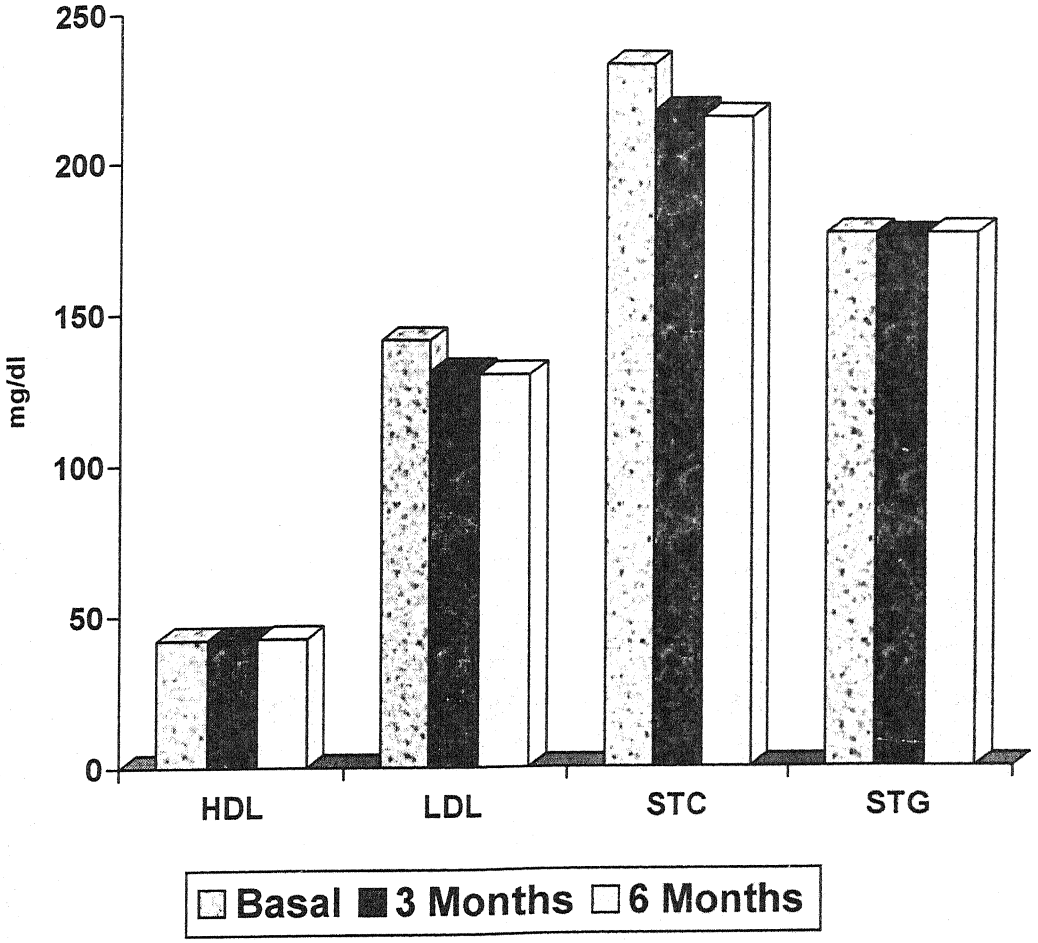


Table - XV

The Effect of treatment on bone mineral density in both the groups after 6 months of therapy.

Bone Mineral Density	Group I				Group II			
	Basal		6 months		Basal		6 months	
	N	%	N	%	N	%	N	%
Normal >0.51gm/cm^3	14	56%	22	88%	13	52%	21	84%
Osteopenia 0.43-0.51gm/cm^3	11	44%	3	12%	12	48%	4	16%
Osteoporosis <0.43gm/cm^3	-	-	-	-	-	-	-	-

On comparing result of bone mineral density in both the groups, it was found that in group I, 11 patients (44%) showed osteopenia at the start of therapy which was reduced to 3 patients (12%) after 6 months of therapy.

Whereas in group II, 12 patients (48%) showed osteopenia at the start of therapy which was reduced to 4 patients (16%) after 6 months of therapy.

These result are comparable in both the groups.

Table - XVI

Comparison of mean values of bone mineral density in both the groups at basal and 6 months of therapy.

Duration of Therapy	Group I (mean ± SD) (gm/cm^2)	Group II (mean ± SD) (gm/cm^2)
basal (a)	.508 ± .197	.501 ± .123
At 6 months (b)	.521 ± .060	.514 ± .116

Applying paired 't' Test to the values shown in the master chart, following results were obtained :-

In Group I

At basal V/s 6 months
a : b, t = 3.88 p < .01 highly Significant

In Group II

At basal V/s 6 months
a : b , t = 3.79 p < .01 highly Significant

On comparing mean values of bone mineral density in table XVI, it was seen that there was significant increase in values of bone mineral density in both the groups.

There is significant increase 2.5% and 2.19% in group I & II respectively.

Comparison of mean values of bone mineral density in both the groups at basal and 6 months of therapy.

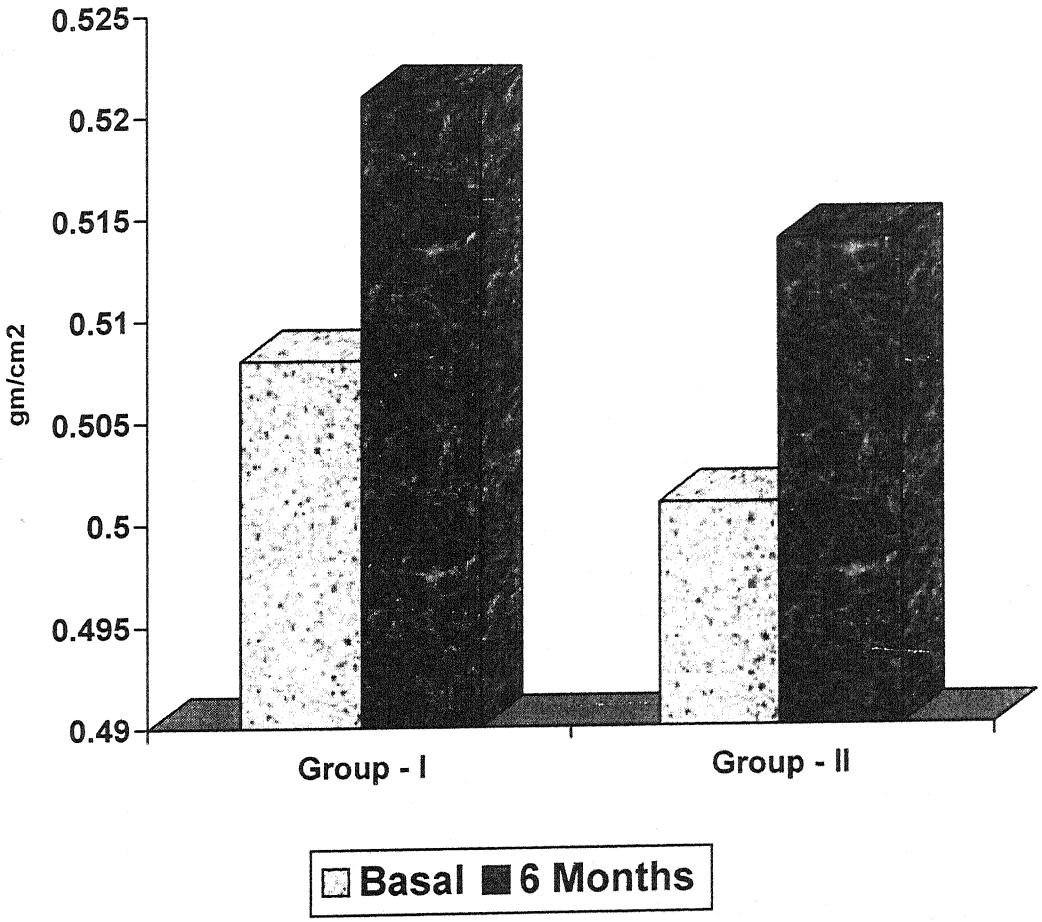


Table - XVII

Comparing results of endometrial biopsy in both the groups at 0 & 6 months of therapy

	Total	0		1		2		3	
		N	%	N	%	N	%	N	%
Baseline									
Group I	11	8	72.72	2	18.18	1	9.1	-	-
Group II	12	11	91.67	1	8.33	-	-	-	-
End Points grades									
Group I	11	1	9.1	1	9.1	7	63.64	2	18.18
Group II	12	10	83.33	2	16.67	-	-	-	-

Table XVII shows that maximum number of patients in both the groups i.e. 72.72% in group I and 91.67% in group II showed no estrogenic effects, at 0 months.

At 6 months, significant moderate to marked estrogenic effects were noted in 81% of estrogen treated patients compared to 0% of Raloxifene treated patients.

Table - XVIII

Showing comparative evaluation of side effects during therapy in both the groups.

Side Effects	Group I		Group II	
	n	%	n	%
Hot flushes	1	4	8	32
Leg cramps	7	28	6	24
Vaginal bleeding	9	36	2	8
Breast pain	8	32	1	4

Table XVIII compares the side effects in both the groups. It shows that incidence of vaginal bleeding was higher in group I (36%) compared to 8% in group I and of breast pain was 32% in group I compared to 4% in group II.

Incidence of hot flushes is much higher in group II i.e. 32% as compared to 4% in group I.

Discussion

DISCUSSION

Today, more and more women are spending about one third of their lives beyond menopause, because of rising average life expectancy for women and this places them at risk for various health problems due to estrogen deficiency.

Both the tissues and organs with estrogen receptors and without estrogen receptors are affected by estrogen so the post menopausal women are at increased risk for cardiovascular disease and osteoporosis. There are still many myths to dispel and facts to discover that could promote a healthier and more productive life style for women of menopausal age group.

Though estrogen, given as replacement therapy, is very effective for symptoms of menopausal syndrome, yet major concern mainly relate to risk of cancer of breast and endometrium due to long term estrogen replacement therapy. So a continuous search is going on for on alternate replacement therapy.

The continuous search has led to discovery of selective estrogen receptor modulators. Many studies have suggested that this approach reduces the LDL level & STG level and improves bone mineral density without having any estrogenic effect on endometrium of post menopausal women. No carcinogenic side effect is reported at optimal doses.

The present study was carried out in the department of Obstetrics and Gynaecology, M.L.B. Medical College, Jhansi (U.P.) to study the effect of Premarin (conjugated equine

estrogen) and Raloxifene (selective estrogen receptor modulator) in post menopausal women.

50 subjects were included in this study and were divided into 2 groups.

In group I :

25 subjects were given oral conjugated equine estrogen (Premarin) Tab. .625 mg/day. Total duration of therapy is 6 months.

In group II :

25 subjects were put on oral tab. Raloxifene 60 mg daily for 6 months. Along with it, calcium tablets were given 500 mg/d.

The lipid profile levels were measured at 0 months and repeated at 3, 6 months. Bone mineral density and endometrial biopsy were done at 0 and 6 months.

Age of Menopause

The age of attainment of menopause in present study i.e. in group I was 40.2 $\pm$ 3.2 years and in group II was 41.7 $\pm$ 1.7 years .

In India, the mean age of attainment of menopause reported by various authors is 42.5 to 48.6 years. (Wyan et al 1966 ;Sharma et al, 1980 ; Sarin et al, 1985 ; Randhawa et al 1987 ; Ankelaria 1995).

So the attainment of menopause in present study was comparable with observations in other studies.

Age of menopause is affected by type of menopause due to increased incidence of panhysterectomies at an early age, undernourishment and smoking.

Duration of Menopause

The duration of menopause in the present study was upto 1 year in 60% in group I and 36% in group II while in 32% in group I and 40% in group II subjects, duration of menopause is 1-3 years.

It was observed that symptoms were more severe in early years as compared to later years of menopause. This finding was confirmed by authors- Utian 1983 and Utian et al 1976.

Nature of Menopause

In the present study, 56% cases in group I and 52% cases in group II had surgical menopause and they were more symptomatic. It shows that women with surgical menopause were more symptomatic when compared with those attaining natural menopause because surgical menopause causes marked reduction in estrogen production abruptly where as in natural process, there is gradual reduction in estrogen production. (Bush et al and Colditz et al, 1987).

Socioeconomic Status

48% subjects in group I and 64% subjects in group II belong to lower middle class. 36% in group I and 20% in group II belong to upper middle class.

Post menopausal System

In present study, vasomotor symptoms like hot flushes 80% in group I and 72% in group II, was the common

symptom. It was followed by palpitations and depressive symptoms (64% in group I and 52% in group II), backache (56% in group I and 44% in group) , joint pains (48% in group I and 32% in group II, dyspareunia (8% in group and 24% in group II). Other symptoms were sweating, insomnia etc.

As observed by Steingold KA et al (1985) , hot flushes were the most common symptom of the menopause that forced subjects to seek medical attention.

The post menopausal symptom in the present study can be compared well with studies by Studd (1992 England) and Anklesaria (1995 from India).

There was marked improvement in hot flushes, sweating, nervousness, palpitations in group I during and after therapy, whereas there was no improvement in these symptoms during and after therapy in group II. In group II, there was slight improvement in symptoms like backache, joint pains etc.

So Raloxifene was not found to be as effective as conjugated equine estrogen in controlling menopausal symptoms. There have been no published studies so far comparing the effects of Raloxifene and premarin on menopausal symptoms.

Effect of Replacement Therapy on lipid profile

High Density Lipoprotein

In the present study, there was increase in HDL levels at 3 months and 6 months in group I as compared to pretreatment values, The increase of 9.31% was statistically significant.

In group II, there was slight increase in HDL level which was statistically insignificant. (p>.05)

The result of present study is comparable with many other studies.

Wahl et al (1983) and Barnes et al (1985) reported an increase of 13.1to 13.4% in HDL level in women treated with conjugated equine estrogen only. The same effect was shown by Miller N.E. (1987), Cust et al (1990), Wolsh et al (1991).

Adami S. et al (1993) studied the effect of oral estrogen on HDL and found significant rise in its levels after treatment.

Draper MW et al (1996) conducted a study comparing the effect of Raloxifene with conjugated equine estrogen and found that HDL-C increased significantly in the estrogen group (16%) but was unchanged in the Raloxifene group.

D. Leo V et al (2001) showed that raloxifene 60mg/day given for 1 year does not produce any changes in HDL-C levels.

Low Density Lipoprotein

In the present study, there was decrease in LDL-C levels at 3 months and 6 months of study as compared to pretreatment levels in both the groups.

In group I , there was 7.72% decrease at 6 months as compared to pretreatment values and it was statistically significant. The decrease in group II was 7.95% and it was also statistically significant. These results were comparable with the results of other authors.

Crook et al (1992) reported reduced serum levels of LDL in groups on oral therapy (both with or without progesterone) of conjugated equine estrogen.

Adami et al (1993) also found significant decrease in total and LDL cholesterol (mean range 6.5 to 18.0%) in subjects on oral therapy of CEE.

Draper MW et al (1996) has shown in his study that LDL-C decreases significantly in the estrogen and raloxifene groups (5-9%).

Walsh BW et al (1998) has shown in his study that Raloxifene at 60 mg/d dosage significantly lowered LDL-C by 12% similar to 14% decrease by HRT.

De leo V et al (2001) demonstrated in his study that mean low density lipoprotein cholesterol levels were lowered by 15% at 12 months of therapy.

Serum total Cholesterol

In the present study, there was decrease in serum total cholesterol levels at 3 months and 6 months as compared to pretreatment levels in both the groups.

The decrease of 7.96% in group I was statistically significant , at the end of 6 months of therapy.

There was decrease of 7.54% in group II and it was also statistically significant.

The findings of the present study correlated well with the following studies -

Crooks et al (1992) observed reduced levels of serum total cholesterol with oral CEE therapy.

Pang et al (1993) had observed a significant decrease in serum cholesterol after oral CEE therapy.

Draper MW et al (1996) demonstrated a decrease of 4-8% in serum total cholesterol level after 6 months of therapy.

Whereas De leo V et al (2001) had observed that serum total cholesterol was reduced by 8.5% after 12 months of therapy with Raloxifene.

Serum Triglycerides

In the present study oral conjugated equine estrogen caused a decrease in serum triglycerides at 3 and 6 months of therapy.

In group I there was statistically significant decrease of 3.11% in STG levels after 6 months of therapy. Whereas there was increase in serum triglycerides of 0.45% after 6 months of therapy in group II. It was not statistically significant.

The findings of serum triglycerides levels can be correlated well with following studies.

Crooks et al (1992) found that oral estrogen therapy increased serum triglycrides by 15%.

Taskinen M.R. et al (1996) studies that serum triglycerides remain unchanged after oral CEE therapy.

A study conducted by Walsh BW et al (1998) demonstrated that triglyceride levels were not changed by Raloxifene but were increased by 20% with HRT.

De Leo V et al in 2001 has found no change in the mean serum triglyceride levels after treatment with raloxifene.

Bone Mineral Density

In the present study, bone mineral density was measured by Dual energy X ray absorbimetry . It was found that 11 subjects i.e. 44% showed osteopenia which was reduced to 12% after 6 months of therapy in group I.

Whereas in group II , 12 subjects (48%) showed osteopenia which was reduced to 16% after 6 months of therapy.

On comparing the mean values in both the groups, there was statistically significant increase of 2.5% in group I and 2.19% in group II.

Felson et al (1993) investigated the effects of 10 years of HRT on BMD in post menopausal women. After 10 years of therapy , bone mineral content was significantly higher in the HRT group when compared to those who received no treatment.

PEPI trial (1996) showed that HRT increases bone density in the spine and hip and produces a reduction in bone turnover.

Schneider et al (1997) demonstrated that estrogen initiated in early post menopausal period and continued into late life is associated with highest bone density.

Khovidhunkit N (1999) has shown in his study that increase in bone mineral density at the spine , total hip, total body has been reported with raloxifene but seems to be less than that seen with estrogen therapy.

Agnusdei D. et al (1999) conducted a study and found that there was a significant increase i.e 2.4% in bone mineral density measured on spine, hip and 2% for over all skeleton.

The results of these studies can be correlated well with the present study which shows that there is significant increase in bone mineral density after 6 months of treatment in both the groups.

Effect on Endometrium

The present study shows that in group I which is treated with conjugated equine estrogen for 6 months, maximum subjects i.e. 72.72% showed no estrogenic effects on endometrial biopsy conducted at the start of therapy , whereas at 6 months, significant moderate to marked estrogenic effects were noted in 81% of subjects.

In group II, at the start of therapy 91.67% of subjects showed no estrogenic effects and after 6 months of treatment with raloxifene, moderate to marked estrogenic effects were not observed in any of the patients.

These findings are comparable to following studies -

Boss SM et al (1997) conducted a 8 week study comparing the effect of raloxifene and estrogen on endometrium. Here at the end point, moderate to marked estrogenic effects were noted in 77% of estrogen treated women versus 0% of raloxifene treated women.

A study conducted by Fugere P t al in 2000 has demonstrated that in the HRT group, 78.7% biopsy specimens showed normal benign post menopausal endometrium, 19.1%

showed benign stimulatory endometrium and 2.1% showed benign abnormal postmenopausal endometrium where as in the raloxifene group, 94.4% showed benign normal post menopausal endometrium and 5.6% were classified as benign stimulatory endometrium.

Side Effects

In the present study , incidence of vaginal bleeding was higher in group I (36%) as compared to group II (8%) and also incidence of breast pain was 32% in group I compared to 4% in group II.

Incidence of hot flushes was much higher in group II i.e. 32% as compared to 4% in group I.

A study conducted by Fugere P et al (2000) is comparable with the present study. At least one instance of vaginal spotting or bleeding was reported by 68% of women in HRT group and by 9% in raloxifene group. Breast pain was reported by 44% in group I and 9% in group II. At least one episode of hot flushes was reported in 31% in group I 3% in group II.

Summary & Conclusion

SUMMARY

The present study was carried out at Department of Obstetrics & Gynaecology , M.L.B. Medical college, Jhansi with an aim to study the effect of hormone replacement therapy versus Raloxifene therapy on postmenopausal symptoms, lipid profile, bone mineral density and endometrial biopsy in subjects with natural or surgical menopause, having post menopausal symptoms.

50 subjects were selected for the study and divided into 2 groups.

Group - I Included 25 subjects who were given oral conjugated equine estrogen (Premarin) .625 mg per day.

Group - II Included 25 subjects who were given Tab. Raloxifene (selective estrogen receptor modulator) 60mg per day along with oral calcium preparation 500mg/day.

History of individual post menopausal symptoms were taken in detail to judge the severity of symptoms and efficacy of both the therapies on different symptoms. These patients underwent thorough general and systemic examination to rule out any dysfunction and to rule out any absolute contra indications. Such patients were excluded from the study specific investigations such as serum lipid profile, bone

mineral density and endometrial biopsy were done at the start of the therapy and then to evaluate the effect of two drugs, lipid profile was repeated at 3 and 6 months. Bone mineral density and Endometrial biopsy were repeated at 6 months.

The following points were observed :-

(a) The mean age of subjects in group I and II was 42.56 ± 3.16 years and 44.20 ± 3.82 years , respectively.

(b) The mean age of attainment of menopause in group I & II was 40.2 ± 3.2 years and 41.7 ± 1.7 years, respectively.

(c) Mean age of attainment of natural menopause was 43.32 ± 2.05 years and mean age of attainment of surgical menopause was 39.32 ± 1.21 years.

(d) Majority of subjects 56% in group I and 52% in group II had surgical menopause.

(e) Vasomotor symptoms like hot flushes (80% in group I and 72% in group II) was commonest symptom followed by palpitation and depressive symptoms (64% in group I and 52% in group II), sweating and psychosomatic symptoms like insomnia, dizziness , headache nervousness ; dyspareunia (8% in group I and 2% in group II). backache and joint pain.

(f) There was marked improvement in hot flushes , sweating, nervousness and palpitations in group I during and after the therapy whereas there was no significant

improvement in these symptoms in group II. There was slight improvement in symptoms like joint pain, backacke etc. in group II.

(g) HDL is a cardioprotective lipoprotein and in present study, 9.31% increase in HDL level was noted after 6 months in group I, which was statistically significant whereas increase of only 1.23% was noted in group II which was statistically insignificant.

(h) In group I subjects, level of LDL-C decreased by 7.72% and this decrease was statistically significant. Almost similar decrease of 7.95% was noted in subjects of group II and was statistically significant.

(i) Statistically significant decrease of 7.96% was observed in the levels of STC in group I after 6 months. A decrease of 7.54% was noted in group II and was also statisitcally significant.

(j) Conjugated equine estrogen administered for 6 months in group I caused a decrease of 3.11% in level of STG which was statistically significant. There was an increase in STG of 0.45% after 6 months of therapy in group II which was statistically insignificant.

(k) On comparing the mean values of bone mineral density in both the groups, there was significant increase of 2.5% in group I and 2.19% in group II.

(l) It was also observed that 44% subjects showed osteopenia in group I at the start of therapy, which was reduced to 12% after 6 months of therapy. In group II, 48% subjects showed osteopenia which was reduced to 16% after 6 months of therapy.

(m) The present study also compared the effect of both the drugs on the endometrium. At the start of therapy, maximum number of subjects in both the groups i.e., 72% in group I and 91% in group II showed no estrogenic effects on endometrial biopsy. At the end of 6 months moderate to marked estrogenic effects were noted in 81% of subjects in group I versus 0% in group II.

(n) Major side effects noted among both the groups were vaginal bleeding, breast pain, leg cramps and hot flushes. Incidence of vaginal bleeding (36%) and breast pain (32%) was much higher among the subjects of group I. Where as that of hot flushes was higher in group II i.e. 32% as compared to 4% in group I.

But these hot flushes experienced by subjects of group II were mild and tolerable. They were more severe, earlier during the therapy becoming milder later on.

In terms of cardiovascular risk, the optimal lipid profile includes elevated HDL levels and reduced LDL levels, serum total cholesterol levels and serum triglyceride level. In our study, HRT shows such effects

But Raloxifene does not increase HDL which is cardioprotective.

In terms of bone mineral density, Raloxifene as well as HRT do increase the BMD in osteopenic subjects, thus reducing the risk of fractures & osteoporosis.

As far as effect on endometrium is concerned, HRT causes estrogenic effects on endometrium, so increasing the risk for endometrial malignancies. But Raloxifene does not cause estrogenic effects on endometrium so it proves to be safer in terms of malignancies.

CONCLUSION

From the present study, it can be concluded that selective estrogen receptor modulator, Raloxifene has not been proved to be as effective as conjugated equine estrogen for controlling post menopausal symptoms but it is well tolerated. Both the therapies have beneficial effects on lipid profile. Although Raloxifene reduced LDL cholesterol and serum total cholesterol, yet no significant changes in HDL cholesterol and triglycerides have been observed, hence confirmation of effect on these factors is still required. The apparently reduced risk of coronary artery disease cannot be attributed with certainity to hormone replacement therapy and raloxifene. It may instead have been caused by healthier life style and medical characteristic of women studied with the possibility that healthier women were selected for the study. Conjugated equine estrogen as well as raloxifene improve the bone mineral density of, osteopenic post menopausal subjects so both might prove to be beneficial in the treatment and prevention of osteoporosis. Raloxifene does not cause proliferation of endometrium, hence it does not predispose to development of uterine malignancy.

Hence the present study concludes that Raloxifene and premarin both are lipid friendly (cardio protective) and have positive effect on BMD. Raloxifene also has favourable effect

on endometrium but at the same time it can not be used as 1st line treatment of post menopausal symptoms for which premarin still remains the drug of choice .

Bibliography

REFERENCE

1. Adami S., Rossini H., Zamberlan N. et al : long term effects of transdermal and oral estrogens on serum lipids and lipoproteins in post menopausal women. Maturitas 1993; 17 : 191-196.
2. Alcysio D, Fabiani AG, Maulani M, Bottiglioni F : Analysis of climacteric syndrome Maturitas 1989 ; 11 : 43 - 53.
3. Agnusdei D, Lin leage S, Augendre Ferrante B: Results of international clinical trials with Raloxifene. Ann. Endocrinology (Paris) 1999 Sep ; 60 (3) : 242-6.
4. Barett CE, Bush TL Estrogen and coronary heart disease in women. JAMA, 1994, 265 : 1861-7.
5. Bengsston C ; Lindquist O : Menopausal effects on risk factors for ischemic heart disease Maturitas 1979 ; 1 : 165-170.
6. Brincat M, Moniz CE, Studd J WW, et al : The longterm effects of menopause and of administration of sex hormones on skin collagen & skin thickness. BJOG 1985 ; 92 : 256-9.
7. Bungay GT, Vessey MI, Mc Phersson GK : study of symptoms in middle life with special reference to the menopause BJOG 1980 ; 281:181-3.
8. Colditz GA, Willett WC, Stampfer MJ, Rosner B, Speizer FE et al : Menopause & risk of coronary heart disease in women. NEJM 1987 ; 316 : 1105 - 1110.
9. Canard J, Basdevant A, Thomas JL etal : cardiovascular risk factors and combined estrogen progesterone replacement Therapy : a

placebo controlled study with nomegestral acetate and estradiol. Fertility and sterility 195 ; 64 : 957-620.

10. Crook D, Cust MP, Gangar XF et al : comparison of transdermal and oral estrogen progesterone replacement therapy, effects on serum lipids and lipoproteins AJOG 1992 ; 166 (3) : 950-55.
11. Campbell S. and whitehead M : Estrogen therapy and menopausal syndrome. Clin. Obs.Gynae 1977 ; 4 : 31-47.
12. Doren M, Reuther G et al : Superior compliance and efficacy of continuous combined oral estrogen-progesterone replacement therapy in post menopausal women. AmJ. Obstet. Gynaecol 1995 ; 173 : 1446-51.
13. Draper MW, Flowers DE, Huster WJ et al : A controlled trial of raloxifene (7139481) HCL : impact on bone turnover and serum lipid profile in healthy post menopausal women J Bone miner Res. 1996 Jun. ; 11 (6) : 835-42.
14. De leo V, La Marca A , Morgante G, Lanzetta D et al : Randomized control study of the effects of raloxifene on serum lipids and homocysteine in older women. Am. J. Obst. Gynaecol 2001 ; 184(3) : 350-53.
15. Erenus M, Kutlay , Pelkin S : comparison of impact of oral various transdermal estrogen on serum lipoprotein. Fertility and sterility 1994 ; 61 : 300-302.
16. Erlik Y, Tatary IV, Meldrom DR et al : Association of waking episodes with hot flushes. JAMA 1981 ; 245 : 1741-44.

17. Ettinger B, Genant HK and Cann CE : Long term estrogen replacement therapy prevent bone loss and fractures Ann. Int. Med. 1985 ; 102 : 319 - 24.

18. Furman RH, Alaupovic P, Howard RP : Effects of anderogens and estrogens on serum lipids and the composition and concentration of serum lipoproteins in normal lipemic and hyperlipidemic states. Prog. Biochem. Pharmacol. 1967 ; 2 : 215-49.

19. Felson DT Zhang , Y. Hannoa MT, Keil DP, Wilson PW & Anderson JJ : Effect of post menopausal estrogen therapy on bone density in elderly women. NEJM , 329 : 1141-1146.

20. Fugere P, Schecle WH, Shah A, Strack TR, Glant MD, Jolly E : uterine effects of raloxifene in comparison with continuous combined hormone replacement therapy in post menopausal women. Am J. Obstet. Gynaecol 2000 ; 182(3) : 568 -74.

21. Gal D, Edman CD, Vellios F et al : Long term effects of megestral acetate in the treatment of endometrial hyperplasia Am J Obtet Gynaecol 1983 ; 146 : 316-22.

22. Gambrell RD JR : The menopause : Benefits and risks of estrogen progestogen replacement therapy.Fertility sterility 1982 ; 37 : 457.

23. Geola FL, Frumar AM, Tataryn IV, Lu KH, Hershman JM et al : Biological effects of various doses of conjugated equine estrogen in postmenopausal women J. of clin. endocrinol & metabolism 1980 ; 51 (3) : 620-5.

24. Gordon T., Castelli WP, Hjortland MC, Kannel WB & Dawber TR : High density lipoprotein as a protective factor against coronary

heart disease. The Framingham study Am. J.Med. 1977 ; 62 : 707 - 714.

25. Gordon T Kanner WB , Hjortland Mc et al : Menopause and coronary heart disease. The framingham study Ann. Intern. Medicine 1978 ; 89 : 157-161.

26. Gordon T, Kanner WB , Bastilli WB. etal : Lipoproteins , Cardiovascular disease and death. The Framingham study Arch Intern. - 4th edition, 1981 ; 141 : 1128.

27. Hill AP, Dworsky R et al : Hormone replacement therapy , hormone levels, lipoprotein cholesterol concentrations in elderly women. AJOG 1996 ; 174 : 897 - 902.

28. Hgstad A and Janson OP : The epidemiology of Climacteric symptoms Acta Obstet. Gynaecol Scarid Supp. 1986; 134 : 59-65.

29. Huppert L.C. (1987) : Hormone replacement therapy, benefits, risks, doses Med. Clin. North Am. 71, 23-29.

30. Handel FE, Geola FL, Meldrum DR et al : Biological effects of various doses of vaginally administered conjugated equine estrogens in post menopausal women. J. Clin. Endo metabol (1983) ; 57 : 133.

31. Kannel W, Hjortland MC, MC Namara PM, Gordon T : Menopause and risk of cardiovascular disease. The Framingham study Ann.. Intern Med. 1976 ; 85 : 447 - 52.

32. Knopp R.H. : Arteriosclerosis risk : Roles of postmenopausal estrogen and oral contraceptives J. Report Med. 1986 ; 31-913.

33. Khovidhunkit W, Shoback DM : Clinical effects of raloxifene hydrochloride in women Ann. Intern Med. 1999 March : 130 (5) : 431 - 9.

34. Lobo R.A. : Clin. Obst. Gynae : 41 : 895 , 1998

35. La Rosa JC : Effects of estrogen replacement therapy on lipid implications for cardiovascular risk. J. Report med 1985 ; 30 (Supply) : 811-3.

36. Lindsay R, Hart et al : The minimum effective dose of estrogen for postmenopausal bone loss. Obstet. Gynaecol (1984) ; 63 ; 759.

37. Lobo R.A. : Cardiovascular implications of estrogen replacement therapy Obstet. Gynecol 75 (Suppl) ; 18-25, 1990.

38. Lobo R.A. et al : Benefits and risk of estrogen replacement therapy AJOG 1995 ; 173 : 982-90.

39. Maltison LA, Cullberg G, Samsioe G : A continuous estrogen - progestogen therapy for climacteric complaints Acta Obstet. Gynaecol Scand 1984 ; 63 : 673-7.

40. Mack TH, Pike MC, Handerson BE et al : Estrogen and endometrial cancer in retirement community NEJM 1976 ; 294 : 1262-1267.

41. Mandel FP, Geola FL,LUJKH, Eggena P et al : Biological effects of various doses of ethinyl estradiol in postmenopausal women Obstetrics & Gynaecology 59 ; 673 - 679, 1982.

42. Mathews KA, Meilahn E, Kuller LH et al : Menopause and risk factors for coronary heart disease NEJM, 1989 ; 321 : 641 - 6.

postmenopausal women Obstetrics & Gynaecology 59 ; 673 - 679, 1982.

42. Mathews KA, Meilahn E, Kuller LH et al : Menopause and risk factors for coronary heart disease NEJM, 1989 ; 321 : 641 - 6.

43. Nachtigall LE, Utian WH : Comparative efficacy and tolerability of transdermal estradiol and conjugated estrogens - a double blind multicentre study. Munchener Medizinische wochen schrift 150 : 28-34, 1988.

44. Novak ER, Woodruff DJ : Novak's gynaecology and obstetrics, pathology with clinical and endocrine relations. Edited by Edmund R Novak, JD Woodruff. Philadelphia. WB Saunders International ,PP. 59-60, 1979.

45. Notelovitz H : Estrogen replacement therapy : Indications contraindications and agent selection. AJOG 1989 ; 161 ; 1832-41.

46. Ottoson V.B. : Oral progesterone and estrogen / progestogen therapy effects of natural and synthetic hormones on subfractions of HDL cholesterol and liver proteins Acta Obstet Gynaecol Scand 1984 ; (Suppl - 127) : 5 - 37.

47. Padwick ML, Endacott J, Whitehead MI : Efficacy acceptability and metabolic effects of transdermal estradiol in the management of postmenopausal women AJOG 152 : 1085-1091, 1985.

48. PEPI Trial group : Effect of estrogen or Esrogen / progesterone regimens on heart disease risk factors in post menopausal women. JAMA , 1985 ; 273 ; 199 - 208.

49. Powers MS, Schenkel L, Darley PE, Good WR, Balestra JC : Pharmacokinetics and pharmacodynamics of transdermal dosage forms of 17 (B) estradiol : Comparison with convertional oral estrogen used for hormone replacement AJOG (152) 1985 ; 1099 - 1106.

50. Pattison NS, Uptin T, Knox B, France J : Transdermal estrogen for postmenopausal women : A double blind cross over comparative study with Ethinyl estradiol. Australian and New Zealand Journal of Obstet and Gynae 29 (1989) ; 62-65.

51. Prentice RL : Tamoxifen as a potential preventive agent in healthy postmenopausal women. Journal of National cancer institute 82. 1310 -1311.

52. Pierre fugere, Wim H. Schecle , Aarti Shah, Thomas R. Strack michael D.Glant, Elaine Jolly : uterine effects of raloxifene in comparison with continuous combined hormone replacement therapy in post menopausal women. Am J. Obstet. Gynaecol 2000 ; 182 : 568-74.

53. Randhawa , Premi HK and Gupta T : The ageof menopause in the women of Himachal Pradesh and the factors affecting the menopause. Indian journal of Public health 1987 ; 1 : 40 - 44.

54. Rosenberg L, Shapiro S, Parmer J : A case control study of myocardial infarction in relation to use of estrogen supplements ,Vol. 137 (1993).

55. Rao SN, Williams T. Cottart J et al : Plasma low density lipoprotein subclasses in coronary 85 peripheral atherosclerosis. Eur J Din . Invest 1983 ; 13 : 18 a (abstra).

56. Rsubin off BE, Wartman J, Rajansky N et al : Effects of hormone replacement therapy on weight , body composition fat distribution and food intake in early post menopausal women : a prospective study. Fertil steril 1995 ; 64 : 963-81.

57. Riggs BL, Jowsey J, Kelley PJ et al : Effect of sex hormone on bone in primary osteoporosis, J din. Invest 1969 ; 48 : 1065-72.

58. Robinson RW , Lebeau RJ : Effect of conjugated equine estrogens on serum lipids and the clotting mechanism. J. Atherosclen Res. 1965 ; 5 : 120-4.

59. Rasano G.M.C. , Chierchia S.L. , Leonardo F. Beale CM, collins P : cardioprotective effects of ovarian hormone Eur. Heart J. 1996 ; 17 (Suppi D) : 15-19.

60. Rogerio A. Lobo : Benefits and risks ofestrogen replacement therapy, AJOG 1995 ; 173 : 982 - 90.

61. Speroff, L.: quality of life issues in the management of menopause , Women and Health Today, Ed. Popkin D. Peddle, L. Newyork : Parthenon , 271-90, 1999.

62. Stanczyk Fz, Shoupe D, Nunez V, Macia Gonazales I, Vijod MA, Lobo RA : A randomized comparison of non oral estradiol delivery in post menopausal women. AJOG 1988 ; 159 : 1540 - 1560.

63. Steingold KA, Lavfer Chetkowski RJ, De Fazio JD, Matt DW et al : treatment of hot flushes with transdermal estradiol administration . Journal of clinical endocrinology and metabolism 1985 ; 61 : 627-32.

64. Studd, JWW, Whitehead M : The menopause Oxford Backwell, 1988.

65. Shaw's text book of Gynaecology ed. V.G. Padubidri, Shirish N. Daftary : Ed. 11 Publishers B.I. Churchill livingstone , New Delhi , 1995.

66. Sherwin BB, Gelfand MM : A prospective one year study of estrogen and progestin is postmenopausal women : Effect on clinical symptoms and lipoproteins , obstet. Gynaecol 1989 ; 73 : 759- 766.

67. Sitruk Ware R. (199Q) ; Estrogen therapy during menopause. Practical Treatment recommendation Drugs 39, 203-217.

68. Smith DC, Prentick , Thomson DJ, Herman WL : Association of exogenous estrogens and endometrial carcinoma. NEJM 1975 ; 293 : 1164.

69. Smith P ; Post menopausal urinary symptoms and HRT BMJ 1976 ; 2 : 941.

70. Smith R, Studd J : Menopause and hormone replacement therapy Martons Quintz Ltd. 1993 ; 1 : 85317.

71. Segurdson G, Nicole A, Lewis B : Conversion of very low density Lipoproteins to lowdensity lipoproteins „ J. Clin. Invest. 1975 ; 56 : 1481.

72. Semmens JP, Wagner G : Estrogen deprivation and vaginal function in post menopausal women, JAMA 1982; 248 : 445.

73. Shattil SL, Anayo Galindo R. Bennet J et al : Platelet hypersenstivity by Cholesterol incorporation, J. Clin. Invest , 1975 ; 55 : 636.

74. Schiff I, Regestin Q., Tuichinsky O, Rayon KJ : Effects of estrogens on sleep and psychological state of hypogonal women. JAMA 1979 ; 242 : 2405.

75. Schiff I, Ryan K.J. : Benefits of estrogen replacement, Obstat Gynaecol Surv 1980 ; 35 : 400.

76. Smith R (1993) Bone mineral in human nutrition and dietetics PP. 162 - 173 (JS Garrow and WPT James - Editors) Edinbourgh, Churchil Livingstone.

77. Susan M. Boss William J Huster Julie A Nelicl , Michael D. Glant , Carol C. Eisenhunt , Michael W. Draper : Effects of Raloxifene Hydrochoride on Endometrium of Post menopausal women. Am J. Obset Gynaecol 1997 : 177 : 1958 - 64.

78. Tataryn IV, Meldrum DK, Frumar AM, Judd HL : LH , FSH and skin temperature during the menopausal hot

flush Journal of Endocrinology RE., Behn BG & Brown BW (1967) : Variation of human menstrual cycle throughout life International journal of fertility 12 : 77 - 126.

79. Taskinen M.R., Puolakka J, Pyorala T, Luotata H et al : Hormone replacement Therapy lowers plasma lipoprotein (a) off spring study : Role of lipoprotein Cholesterol. Am J. Cardiol 1980 : 46 : 649 Am J Obst. Gynaecol 1987, 156 : 1335 - 38.

80. Thompson ER, Teng B & Sviderman : Metabolic conversion of light to heavy low density (LDL) in subjects with normal and increased plasma LDL - Apo B levels circulation 1981 ; 66 (Suppl) 1174 (Obst.)

81. Utian WH : Mrnopause in modern perspective, New York, Appleton century Crofts , 1980.

82. Utian WH : The symptom complex associated with the menopause semin Report Endocrinol 1983 ; 1 : 1.

83. Utian WH : Biosynthesis and physiologic effects of estrogen and pathophysiologic effects of estrogen deficiency:A review Am. J.Obst.Gynae.1989;161:1828-31.

84. Usha Krisna, Rajeev V. Mehtra : Osteoporosis-Incidence and Implications, J. Obstetrics & Gynaecol of India, Vol. 50, No. 5 Oct. 2000.

85. Vincenzo De Leo , Antonio la marca, Giuseppe Morgante, Danila Lanzetta, Carlo setacci, Felice Petraglia : Randomized control study of the effects of raloxifene on

serum lipids and homocysteine in older women. AJOG 2001 ; 184 : 350 -3.

86. Weiss NS, Ure CL, Bullard JA, et al : decreased risk of fractures of the hip and lower forearm with post menopausal use of estrogen NEJM 1980 ; 303 : 1195-8.

87. Wilson PW, Garrison RJ, Castellis WP et al : Prevalence of post menopausal coronary heart disease in the framingham off spring study : Role of lipoprotein Cholesterol Am J. Cardiol 1980 ; 46 : 649 Am J. Obst. Cynacol 1987, 156 : 1335-38.

88. Wahl P. Walden C Knoop R et al : Effect of estrogen / progestin potency on lipid lipoprotein cholesterol, NEJM, 1983 ; 308 : 862 - 67.

89. Wallace RB, Hoover J, Barret connor E, Rifkind BM, Huninghake DB, Mackentheun A and Heiss G : Altered plasma lipid and lipoprotein levels associated with oral contraceptive and estrogen use lancet 1979 ; 111-115.

90. Wallace RB, Anderson Rh : Blood lipids, lipids related measure and the risk of atherosclerotic cardiovascular disease Epidemol Rev. 1987 ; 9 : 95.

91. Walsh BW, Schiff I, Rosner B. et al : Effects of post menopausal estrogen replacement of the concentrations and metabolism of plasma lipoproteins NEJM , 1991 : 325 : 1196-1204.

92. Weinstein L : Efficacy of continuous estrogen progestin regimen in menopausal ratient.

93. Wilcox JN, Blumenthal BF (1995) : Thrombotic mechanism in atherosclerosis Journal of Nutrition 125 : 631 - 638.

94. Wolfe BM & Huff (1995) : Effects of continuous low dosage hormone replacement therapy on lipoprotein metabolism in postmenopausal women. Metabolism 44 : 410 - 417.

95. WHO 194 assessment of fracture risk and its application for screening for post menopausal osteoporosis Technical Report series No. 834 Geneva WHO.

Master Chart

Group I

A	B	C	D	E	F	G	H	I		J			K			L			M			N		O		P
								1	2	1	2	3	1	2	3	1	2	3	1	2	3	1	2	1	2	
1.	48 Yr.	N	44 Yr	5 Yr.	P_3	UM	U	Hf, Bac ↑ FOM Sw.Jp	JP. BAC.	35	36	42	154	144	143	240	200	218	182	208	210	.452	.582	0	1	VB,L
2.	42 Yr.	S	41 Yr	1 Yr.	P_4	LM	R	Hf. Dys. Par.Bac Diz	Dys. Inc	48	48	47	160	143	142	220	170	200	156	140	140	.632	.640	-	-	L
3.	45 Yr.	N	43 Yr	2 Yr.	P_3	LM	R	Sw.Fat. Ins. Pal. Bac ↑ FOM	↑ FOM	48	48	48	155	143	142	210	250	200	185	208	210	.433	.433	0	2	VB
4.	40 Yr.	S	39 Yr	1 Yr.	P_3	UM	U	↑FOM Jp Dep. Bac ↑ FOM	Cp. Diz.	47	48	50	155	143	142	178	250	170	165	156	150	.586	.590	-	-	BP
5.	42 Yr.	N	40 Yr	2 Yr.	P_4	L	R	Jp Bac. Dep. Pal ↑ FOM		44	44	43	151	143	142	200	240	250	165	166	166	.533	.542	0	0	-
6.	43 Yr.	N	41 Yr	2 Yr.	P_3	LM	R	Hf. w.Ner. FOM Dep.		44	44	39	148	142	142	240	169	250	178	166	166	.611	.623	1	2	VB
7.	40 Yr.	S	39 Yr	1 Yr.	P_3	LM	R	Hf. Bac. FOM Pal.	Diz.	41	41	46	152	126	126	250	200	240	180	166	196	.422	.432		-	L
8.	48 Yr.	N	45 yr	3 Yr.	P_3	LM	R	Sw. Jp Bac. Dep. FOM	-	33	33	42	150	145	136	220	200	169	168	196	164	.500	.582	0	2	VB
9	42 Yr.	N	40 Yr	2 Yr.	P_4	LM	R	Hf. Wg. Dep. Pal.	Jp. Wg.	33	33	40	156	146	137	210	210	200	158	164	145	.532	.548	0	3	VB
10.	40 Yr.	S	39 Yr	1 Yr.	P_3	UM	U	Hf. Fat. Ner. Bec Pal.	-	33	33	44	124	148	139	220	200	200	150	147	138	.468	.588	-	-	Hf
11.	39 Yr.	S	44 Yr	6 Yr.	P_3	UM	R	Hf. Ins. Par. Hec.	-	47	47	48	126	121	120	226	176	210	170	138	160	.576	.576	-	-	L
12.	41 Yr.	S	22 Yr	8 Yr.	P_4	LM	U	Hf. Wg. Hec. Sw. Jp. Ner	↑ FOM	46	46	46	153	124	124	210	220	200	184	161	169	.598	.600	-	-	BP
13.	46 Yr.	S	38 Yr	1 Yr.	P_3	UM	R	Hf. FOM Dep. Bac Sw.	Jp. Bac.	43	43	44	153	138	138	184	200	176	178	171	170	.466	.596	-	-	BP
14.	42 Yr.	S	44 Yr	10 Yr.	P_4	UM	R	Hf. Sw. Pal. Dep. Hec. Ner.	Jp	42	42	45	148	146	136	230	200	210	158	170	148	.621	.630	-	-	L,BP
15.	48 Yr.	S	49 Yr	1 Yr.	$P_4 A_1$	UM	U	Hf. Fom Dep Ins.		40	40	49	152	141	145	210	220	200	178	148	170	.586	.600	-	-	-
16.	42 Yr.	N	41 Yr	3 Yr.	P_4	LM	R	Jp.Bac.Fat. Diz.Dep.	-	48	48	48	152	138	141	210	160	200	165	170	166	.476	.482	1	3	VB

17.	41 Yr.	S	46 Yr	1 Yr.	P_5	UM	R	Jp.Bac.Fat. Diz.Dep.	-	38	38	42	140	142	130	246	220	218	140	155	126	.550	.550	-	-	BP
18.	43 Yr.	N	46 Yr	>5 Yr.	P_2	LM	U	Hf. FOM Sw. Pal. Dys.	-	49	49	50	146	136	133	178	160	160	120	126	103	.463	.484	0	3	VB
19.	42 Yr.	S	44 Yr	1 Yr.	P_3	LM	U	Hf.Sw.Pal.Par.	-	40	40	50	136	129	134	242	220	220	170	103	148	.546	.549	-	-	L;BP
20.	42 Yr.	S	45 Yr	10 Yr.	P_2A_1	LM	U	Hf.Ner.Bac. Dep. Pal.Fat.	Jp	38	38	38	132	150	120	100	110	170	180	150	195	.567	.576	-	-	VB
21.	41 Yr.	N	41 Yr	2 Yr.	P_4	LM	U	Hf.Dp.Dep.Pal. Sw.	-	30	32	39	152	147	148	210	200	200	178	194	136	.456	.573	0	2	VB
22.	39 Yr.	S	40 Yr	14 Yr.	P_4	LM	R	Hf.Jp.Pal.Sw.	-	30	31	41	150	156	146	200	180	178	148	136	136	.588	.598	-	-	BP
23.	46 Yr.	S	45 Yr	10 Yr.	P_5	L	R	Pal.Jp.Dep.Bec. Sw.	-	39	39	40	160	126	147	226	220	220	150	136	167	.546	.560	-	-	L
24.	48 Yr.	N	45 Yr	2 Yr.	P_4A_2	L	R	Hf.Bac.Ner.Hec. PAl.	-	41	41	42	148	126	126	224	220	218	165	136	182	.489	.409	0	2	BP
25.	41 Yr.	S	43 Yr	1 Yr.	P_4	LM	U	Hf. Jp. Bac. Ner. Pal.	Jp. Bac	33	34	45	130	126	126	250	240	238	170	166	154	.450	.596	2	2	VB

Group II

A	B	C	D	E	F	G	H	I		J			K			L			M			N		O		P
								1	2	1	2	3	1	2	3	1	2	3	1	2	3	1	2	1	2	
1.	48 Yr	N	46 Yr	1.5 Yr	P_3	L	R	Hf, Pal, Dep	JP. CP	45	46	46	124	120	120	240	230	230	178	180	182	.539	.542	0	0	V_B
2.	40 Yr	S	39 Yr	10 Yr	P_3	LM	U	Hf, Pal, Dep Sw, Fat	-	45	45	46	134	130	130	240	220	220	178	172	174	.581	.581	-	-	Hg
3.	46 Yr	N	41 Yr	4.5 Yr	P_4	LM	U	Hf,Pal,Dep↑ Sw, Fat	↑ FOM	36	35	36	138	130	130	248	210	210	170	180	182	.562	.566	0	0	BP,L
4.	45 Yr	N	43 Yr	2 Yr	P_4	LM	R	Pal,Sw, Ner, FOM	Sw, Dys	38	38	40	110	96	96	248	210	210	176	182	184	.597	.597	0	0	-
5.	50 Yr	N	45 Yr	>5 Yr	P_4	LM	R	Hf, Sw, Dep Dys.	He	38	38	38	130	110	110	240	210	200	156	160	160	.583	.583	0	0	V_B
6.	41 Yr	S	40 Yr	10 Yr	P_4	LM	U	Hf, Sw, Dep FOM	Ins, Dys	39	39	39	126	110	110	250	230	230	168	184	184	.502	.632	-	-	Hf
7.	46 Yr	N	42 Yr	4 Yr	P_3	LM	U	Hf, Bac, Pal.↑ FOM	Bac	41	41	42	126	118	112	248	240	242	202	198	198	.482	.566	0	1	-
8.	45 Yr	N	42 Yr	3 Yr	P_4	LM	U	Hf, Dep, Pal, ↑ FOM	-	43	44	45	140	126	126	248	224	222	168	164	164	.531	.535	0	0	L
9.	40 Yr	S	39 Yr	1 Yr	P_3	LM	U	Hf. Dep, Pal, ↑ FOM	Jp Wg	32	33	34	138	120	122	246	220	220	180	190	190	.510	.543	-	-	Hf L
10.	42 Yr	S	40 Yr	2 Yr	$P_3 A_1$	UM	U	Jp. Bac. Dys	Jp.	38	38	38	134	126	126	240	222	220	175	167	170	.567	.567	-	-	-
11.	40 Yr	S	39 Yr	1 Yr	P_3	UM	R	Hf.Ins.If.Bac N ef ↑FOM	-	43	43	45	124	112	112	248	215	215	176	180	182	.539	.539	-	-	-
12.	48 Yr	N	44 Yr	3.5 Yr	P_4	LM	R	Hf Fat.He.Jp. Di z ↑ FOM	-	45	45	45	126	120	120	248	220	218	170	160	164	.432	.500	0	0	Hf
13.	43 Yr	N	40 Yr	3 Yr	P_4	UM	U	Hf,Pal.Bac. Ins ↑ FOM	He	45	45	46	136	128	119	250	250	230	178	160	162	.527	.537	0	0	L
14.	38 Yr	S	37 Yr	6 Yr	P_4	UM	U	Hf Bac Pal Ins Bac	Bac	31	32	34	150	139	139	200	180	180	196	204	206	.432	.462	-	-	-
15	41 Yr	S	40 Yr	6 Yr	$P_3 A_1$	UM	U	Hf↑ FOM Sw B ac Fat	-	39	39	40	154	140	140	240	212	210	182	170	192	.441	.468	-	-	Hf
16.	39 Yr	S	38 Yr	8 Yr	P_3	LM	U	Hf Dep Jp Bac. Pal	Jp. Bac	42	42	44	150	140	140	248	210	210	190	200	200	.503	.633	-	-	-
17.	50 Yr	N	46 Yr	4 Yr	P_4	UM	U	Hf Sw PalBac Ins	-	43	43	45	156	140	140	250	220	220	184	174	170	.568	.570	0	0	-

	Yr		Yr					Ins																	
18.	48 Yr	N	46 Yr	2 Yr	P_3	LM	R	Hf.Sw.Pal.Jp P ar.Ins↑FOM	Jp. Ins	48	48	48	151	145	145	248	220	220	200	202	200	.500	.682	0	0	Hf
19.	47 Yr	N	44 Yr	3 Yr	P_4	LM	R	Hf. Pal.Bac. Dys ↑ FOM	Bac	41	42	44	202	196	187	252	250	248	200	180	190	.502	.630	1	1	Hf
20.	40 Yr	S	39 Yr	1 Yr	P_4	L	R	Dep.Jp.Ner. Pal Ins	Jp	45	45	46	140	134	134	224	214	210	180	184	186	.552	.552	-	-	-
21.	42 Yr	S	41 Yr	8 Yr	P_4	LM	U	Cp.Sw.Dys. De. p.	Cp. Dys	44	45	6	154	149	146	234	220	222	155	140	142	.562	.564	-	-	-
22.	42 Yr	S	40 Yr	2 Yr	P_4	LM	R	Ins. Pal .Hf. Bac	-	53	54	56	152	140	140	230	210	208	152	142	142	.539	.540	-	-	L
23.	50 Yr	N	45 Yr	4.5 Yr	P_2	LM	R	Dep. Bac. Pal. In s.	Diz	55	55	55	150	136	136	248	226	220	175	180	180	.578	.588	0	0	
24.	48 Yr	N	45 Yr	3 Yr	P_3	LM	R	Ins. ↑ FOM sw F at Pal.	-	42	43	43	149	138	138	198	170	170	165	160	162	.444	.568	0	0	
25.	46 Yr	N	43 Yr	3 Yr	P_2	LM	R	Hf Jp. Dep. Bec.	-	44	42	42	140	135	135	250	220	220	178	180	176	.444	.469	0	0	-

'A' for	Serial Number
'B' for	Age of Subjects
'C' for	Mode of menopause
'D' for	Age of onset of menopause
'E' for	Duration of menopause
'F' for	Parity
'G' for	Socio Economic Status
'H' for	Population of subjects (Urban or Rural)
'I' for	I_1 , Symptoms of subjects before therapy I_2 , Symptoms of subjects after 6 months of therapy
'J' for	J_1 , Basal HDL (mg/dl) J_2 , HDL after 3 months (mg/dl) J_3 , HDL after 6 months (mg/dl)
'K' for	K_1 , Basal LDL (mg/dl) K_2 , LDL after 3 months (mg/dl) K_3 , LDL after 6 months (mg/dl)
'L' for	L_1 , Basal Serum total cholesterol (STC) (mg/dl) L_2 , STC after 3 months (mg/dl) L_3 , STC after 6 months (mg/dl)
'M' for	M_1 , Basal Serum Triglyceride (STG) (mg/dl) M_2 , STG after 3 months (mg/dl) M_3 , STG after 6 months (mg/dl)
'N' for	N_1 , Basal bone mineral density (BMD) (gm/cm^2) N_2 , BMD after 6 months (gm/cm^2)
'O' for	O_1 , Basal Endometrial biopsy grade O2, Endometrial biopsy grade after 6 months
'P' for	Adverse effects